

1078/1B925US1

Colon Cancer KH-1 and N3 Antigens

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This application is based on U.S. Provisional Application Serial No. 60/034,950, filed January 13, 1997, the contents of which are hereby incorporated by reference into this application. This invention was made with government support under grants CA-28824-18, GM-15240-02, GM-16291-01, HL-25848-14 and AI-16943 from the National Institutes of Health. Accordingly, the U.S. Government has certain rights in the invention.

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Throughout this application, citations for various publications are provided. The disclosures of these publications are hereby incorporated in their entirety by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Field of the Invention

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The present invention is in the field of tumor-specific cell-surface antigens. In particular, the present invention relates to processes for the preparation of KH-1 and N3 antigens and analogues thereof which are useful as anticancer therapeutics. The present invention also provides novel compositions of matter which serve as intermediates for preparing the KH-1 and N3 antigens.

Background of the Invention

The function of carbohydrates as structural materials and as energy storage units in biological systems is well recognized. By contrast, the role of carbohydrates as signaling molecules in the context of biological processes has only recently been appreciated. (M. L. Phillips, E. Nudelman, F. C. A. Gaeta, M. Perez, A. K. Singhal, S. Hakomori, J. C. Paulson, *Science*, 1990, 250, 1130; M. J. Polley, M. L. Phillips, E. Wagner, E. Nudelman, A. K. Singhal, S. Hakomori, J.C. Paulson, *Proc. Natl. Acad. Sci. USA*, 1991, 88, 6224; T. Taki, Y. Hirabayashi, H. Ishikawa, S. Kon, Y. Tanaka, M. Matsumoto, *J. Biol. Chem.*, 1986, 261, 3075; Y. Hirabayashi, A. Hyogo, T. Nakao, K. Tsuchiya, Y. Suzuki, M. Matsumoto, K. Kon, S. Ando, *ibid.*, 1990, 265, 8144; O. Hindsgaul, T. Norberg, J. Le Pendu, R. U. Lemieux, *Carbohydr. Res.*, 1982, 109, 109; U. Spohr, R.U. Lemieux, *ibid.*, 1988, 174, 211)

The elucidation of the scope of carbohydrate involvement in mediating cellular interaction is an important area of inquiry in contemporary biomedical research. The carbohydrate molecules, carrying detailed structural information, tend to exist as glycoconjugates (cf. glycoproteins and glycolipids) rather than as free entities. Given the complexities often associated with isolating the conjugates in homogeneous form and the difficulties in retrieving intact carbohydrates from

5 these naturally occurring conjugates, the applicability
of synthetic approaches is apparent. (For recent reviews
of glycosylation see: Paulsen; H., *Angew Chem. Int. Ed.*
Engl., 1982, 21, 155; Schmidt, R. R., *Angew. Chem. Int.*
10 *Ed. Engl.*, 1986, 25, 212; Schmidt, R. R., *Comprehensive*
Organic Synthesis, Vol. 6, Chapter 1(2), Pergamon Press,
Oxford, 1991; Schmidt, R. R., *Carbohydrates, Synthetic*
Methods and Applications in Medicinal Chemistry, Part I,
Chapter 4, VCH Publishers, Weinheim, New York, 1992. For
the use of glycals as glycosyl donors in glycoside
15 synthesis, see Lemieux, R. U., *Can. J. Chem.*, 1964, 42,
1417; Lemieux, R. U., Faser-Reid, B., *Can. J. Chem.*,
1965, 43:1460; Lemieux, R. U., Morgan, A. R., *Can. J.*
Chem., 1965, 43, 2190; Thiem, J., Karl, H., Schwentner,
J., *Synthesis*, 1978, 696; Thiem, J. Ossowski, P.,
20 *Carbohydr. Chem.*, 1984, 3, 287; Thiem, J., Prahst, A.,
Wendt, T. *Liebigs Ann. Chem.*, 1986, 1044; Thiem, J., in
Trends in Synthetic Carbohydrate Chemistry, Horton, D.,
Hawkins, L. D., McGarvey, G. L., eds., ACS Symposium
Series #386, American Chemical Society, Washington, DC,
25 1989, Chapter 8.)

The carbohydrate domains of the blood group
substances contained in both glycoproteins and
glycolipids are distributed in erythrocytes, epithelial
cells and various secretions. The early focus on these
30 systems centered on their central role in determining
blood group specificities. (R. R. Race and R. Sanger,
Blood Groups in Man, 6th ed., Blackwell, Oxford, 1975)
However, it is recognized that such determinants are

5 broadly implicated in cell adhesion and binding
phenomena. (For example, see M. L. Phillips, E. Nudelman,
F. C. A. Gaeta, M. Perez, A. K. Singhal, S. Hakomori, J.
C. Paulson, *Science*, 1990, 250:1130.) Moreover,
ensembles related to the blood group substances in
10 conjugated form are encountered as markers for the onset
of various tumors. (K. O. Lloyd, *Am. J. Clinical Path.*,
1987, 87, 129; K. O. Lloyd, *Cancer Biol.*, 1991, 2:421)
Carbohydrate-based tumor antigenic factors might find
applications at the diagnostic level, as resources in
15 drug delivery or ideally in immunotherapy. (Toyokuni, T.,
Dean, B., Cai, S., Boivin, D., Hakomori, S., and Singhal,
A. K., *J. Am. Chem Soc.*, 1994, 116, 395; Dranoff, G.,
Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H.,
Brose, K., Jackson, V., Hamada, H., Paardoll, D.,
20 Mulligan, R., *Proc. Natl. Acad. Sci. USA*, 1993, 90, 3539;
Tao, M. H., Levy, R., *Nature*, 1993, 362, 755; Boon, T.,
Int. J. Cancer, 1993, 54, 177; Livingston, P. O., *Curr.*
Opin. Immunol., 1992, 4, 624; Hakomori, S., *Annu. Rev.*
Immunol., 1984, 2, 103; K. Shigeta, et al., *J. Biol.*
25 *Chem.*, 1987, 262, 1358)

The use of synthetic carbohydrate conjugates to
elicit antibodies was first demonstrated by Goebel and
Avery in 1929. (Goebel, W. F., and Avery, O. T., *J. Exp.*
Med., 1929, 50, 521; Avery, O. T., and Goebel, W. F., *J.*
30 *Exp. Med.*, 1929, 50, 533.) Carbohydrates were linked to
carrier proteins via the benzenediazonium glycosides.
Immunization of rabbits with the synthetic antigens
generated polyclonal antibodies. Other workers (Allen,

5 P. Z., and Goldstein, I. J., *Biochemistry*, 1967, 6, 029;
Rude, E., and Delius, M. M., *Carbohvdr. Res.*, 1968, 8,
219; Himmelspace, K., et al., *Eur. J. Immunol.*, 1971, 1,
106; Fielder, R. J., et al., *J. Immunol.*, 1970, 105, 265)
developed similar techniques for conjugation of
10 carbohydrates to protein carriers. Most of them suffered
by introducing an antigenic determinant in the linker
itself, resulting in generation of polyclonal antibodies.
Kabat (Arakatsu, Y., et al., *J. Immunol.*, 1966, 97, 858),
and Gray (Gray, G. R., *Arch. Biochem. Bioshys.*, 1974,
15 163, 426) developed conjugation methods that relied on
oxidative or reductive coupling, respectively, of free
reducing oligosaccharides. The main disadvantage of
these techniques, however, is that the integrity of the
reducing end of the oligosaccharide was compromised. In
20 1975 Lemieux described the use an 8-carbomethoxy-1-
octanol linker (Lemieux, R. U., et al., *J. Am. Chem.*
Soc., 1975, 97, 4076) which alleviated the problem of
linker antigenicity and left the entire oligosaccharide
intact. Equally effective in producing glycoconjugates
25 was the allyl glycoside method described by Bernstein and
Hall. (Bernstein, M. A., and Hall, L. D., *Carbohydr.*
Res., 1980, 78, Cl.) In this technique the allyl
glycoside of the deblocked sugar is ozonized followed by
a reductive workup. The resultant aldehyde is then
30 reductively coupled to a protein carrier with sodium
cyanoborohydride.

In the mid-70's and early 80's Lemieux and his
collaborators made contributions to antibody production

5 stimulated by synthetic glycoconjugates (Lemieux, R. U.,
et al., *J. Am. Chem. Soc.*, **1975**, 97, 4076) and to
conformational issues (Lemieux, R. U., et al., *Can. J.*
Chem., **1979**, 58, 631; Spohr, U., et al., *Can. J. Chem.*,
10 **1985**, 64, 2644; Vandonselaar, M., et al., *J. Biol. Chem.*,
1987, 262, 0848) important in the interactions of the
blood group determinants (and analogues thereof) with
the carbohydrate binding proteins known as lectins. More
recently, workers at Bristol-Myers Squibb reported the X-
ray crystal structure of the Lewis y epitope complexed
15 with the antibody BR96. (Jeffrey, P. D., et al., *Nature*
Structural Biol., **1995**, 2, 466.) Two main components
appear to govern recognition between carbohydrates and
most antibodies. The first is multiple hydrogen bonding
between the sugar hydroxyls and the amino acid residues
20 of Asp, Asn, Glu, Gln, and Arg. The second major
interaction is stacking between the sugar-ring faces and
aromatic side chains, which occurs most frequently with
tryptophan. In the complex with BR96 the most
significant interactions involve the latter; additional
25 hydrogen bonding occurs between the sugar hydroxyls and
the indole nitrogens. Most antibody binding sites can
support about 6 linear carbohydrate residues in a groove
or cavity shaped binding site.

Glycoconjugates may be used in direct
30 immunotherapy or the monoclonal antibodies generated from
vaccinations may be used to specifically target known
chemotherapeutic agents to tumor sites. The immune
response to carbohydrates is generally not strong,

5 resulting mainly in production of IgM type antibodies.
IgM antibodies are capable of complement fixation.
Complement is a family of enzymes that can lyse cells to
which antibodies are bound. The response to carbohydrate
10 antigens normally does not enlist the use of T-cells
which would aid in the body's rejection of the tumor.
While the probability of complete tumor rejection as a
result of vaccination with a conjugate is unlikely, such
treatments will boost immune surveillance and recurrence
of new tumor colonies can be reduced. (Dennis, J., *Oxford*
15 *Glycosystems Glyconews Second, 1992*; Lloyd, K. O., in
Specific Immunotherapy of Cancer with Vaccines, 1993, New
York Academy of Sciences, 50-58.) Toyokuni and Singhal
have described a synthetic glycoconjugate (Toyokuni, T.,
et al., *J. Am. Chem. Soc., 1994, 116, 395*) that
20 stimulated a measurable IgG titer, a result which is
significant since an IgG response is generally associated
with enlistment of helper T cells.

The use of immunoconjugates has shown promise
in the reduction of large tumor masses. The workers at
25 Bristol-Myers Squibb (Trail, P. A., et al., *Science*,
1993, 261, 212) have described the attachment of the
known chemotherapeutic drug doxorubicin to the antibody
BR96. BR96 is an anti-Lewis y antibody which has been
shown to bind to human breast, lung and colon carcinomas.
30 Athymic mice that have had human cancers (L2987-lung,
RCA-colon, and MCF7-breast carcinomas) xenografted
subcutaneously were treated with the drug-antibody
conjugate (BR96-DOX). The result was complete regression

5 of the tumor mass in 78% of the mice treated. BR96 is
efficiently-internalized by cellular lysosomes and
endosomes following attachment to the cell surface. The
change in pH upon internalization results in cleavage of
the labile hydrazone thereby targeting the drug
10 specifically to the desired site.

Many of the blood group determinant structures
can also occur in normal tissues. Antigen expression in
normal cells and cancer cells can have subtle
distributional differences. In the case of Le y, which
15 does appear in normal tissues, the expression of the
determinant in tumor cells tends to be in the form of
mucins which are secreted. Mucins are glycoproteins with
a high content of the amino acids serine and threonine.
It is through the hydroxyl functionality of these amino
20 acids that Lewis y is linked. Thus, in terms of
generating competent antibodies against tumor cells
expressing the Le y antigen, it is important that the
antibody recognize the mucin structure.

Structurally, the blood group determinants fall
25 into two basic categories known as type I and type II.
Type I is characterized by a backbone comprised of a
galactose 1-3 β linked to N-acetyl glucosamine while type
II contains, instead, a 1-4 β linkage between the same
building blocks (cf. N-acetyl lactosamine). The position
30 and extent of a-fucosylation of these backbone structures
gives rise to the Lewis-type and H-type specificities.
Thus, monofucosylation at the C4-hydroxyl of the N-acetyl
glucosamine (Type I series) constitutes the Le a type,

5 whereas fucosylation of the C3-hydroxyl of this sugar
 (Type II series) constitutes the Le x determinant.
 Additional fucosylation of Le a and Le x types at the C2'
 hydroxyl of the galactose sector specifies the Le b and
 Le y types, respectively. The Le y determinant is
10 expressed in human colonic and liver adenocarcinomas.
 (Leverly, S. B., et al., *Carbohydr. Res.*, 1986, 151, 311;
 Kim, Y. S., *J. Cellular Biochem. Suppl.*, 16G 1992, 96;
 Kaizu, T., et al., *J. Biol. Chem.*, 1986, 261, 11254;
 Leverly, S. B., et al., *Carbohydr. Res.*, 1986, 151, 311;
15 Hakomori, S., et al., *J. Biol. Chem.*, 1984, 259,
 4672;Fukushi, Y., et al., *ibid.*, 1984, 259, 4681;
 Fukushi, Y., et al., *ibid.*, 1984, 259, 10511.)

 The presence of an α -monofucosyl branch, solely
 at the C2'-hydroxyl in the galactose moiety in the
20 backbone, constitutes the H-type specificity (Types I and
 II). Further permutation of the H-types by substitution
 of α -linked galactose or α -linked N-acetylgalactosamine
 at its C3'-hydroxyl group provides the molecular basis of
 the familiar serological blood group classifications A,
25 B, and O. (Lowe, J. B., *The Molecular Basis of Blood
 Diseases*, Stamatoyannopoulos, et al., eds., W. B.
 Saunders Co., Philadelphia, PA, 1994, 293.)

 Several issues merit consideration in
 contemplating the synthesis of such blood group
30 substances and their neoglycoconjugates. For purposes of
 synthetic economy it would be helpful to gain relief from
 elaborate protecting group manipulations common to
 traditional syntheses of complex branched carbohydrates.

5 Another issue involves fashioning a determinant linked to
a protein carrier. It is only in the context of such
conjugates that the determinants are able to galvanize B-
cell response and complement fixation. In crafting such
10 spacer units between the carbohydrate determinant and the
carrier. (Stroud, M. R., et al., *Biochemistry*, 1994, 33,
0672; Yuen, C. T., et al., *J. Biochem.*, 1994, 269, 1595;
Stroud, M. R., et al., *J. Biol. Chem.*, 1991, 266, 8439.)

The present invention provides new strategies
15 and protocols for oligosaccharide synthesis. The object
is to simplify such constructions such that relatively
complex domains can be assembled with high stereo-
specificity. Major advances in glycoconjugate synthesis
require the attainment of a high degree of convergence
20 and relief from the burdens associated with the
manipulation of blocking groups. Another requirement is
that of delivering the carbohydrate determinant with
appropriate provision for conjugation to carrier proteins
or lipids. (Bernstein, M.A., and Hall, L. D., *Carbohydr.*
25 *Res.*, 1980, 78, C1; Lemieux, R. U., *Chem. Soc. Rev.*,
1978, 7, 423; R. U. Lemieux, et al., *J. Am. Chem. Soc.*,
1975, 97, 4076.) This is a critical condition if the
synthetically derived carbohydrates are to be
incorporated into carriers suitable for biological
30 application.

Antigens which are selective or ideally
specific for cancer cells could prove useful in fostering
active immunity. (Hakomori, S., *Cancer Res.*, 1985, 45,

5 2405-2414; Feizi, T., *Cancer Surveys*, 1985, 4, 245-269)
Novel carbohydrate patterns are often presented by
transformed cells as either cell surface glycoproteins or
as membrane-anchored glycolipids. In principle, well
chosen synthetic glycoconjugates which stimulate antibody
10 production could confer active immunity against cancers
which present equivalent structure types on their cell
surfaces. (Dennis, J., *Oxford GlycoSystems Glyconews*
Second, 1992; Lloyd, K. O., in *Specific Immunotherapy of*
Cancer with vaccines, 1993, New York Academy of Sciences
15 pp. 50-58) Chances for successful therapy improve with
increasing restriction of the antigen to the target cell.
A glycosphingolipid was isolated by Hakomori and
collaborators from the breast cancer cell line MCF-7 and
immun characterized by monoclonal antibody MBrl.
20 (Bremer, E. G., et al., *J. Biol. Chem.*, 1984, 259, 14773-
14777; Menard, S., et al., *Cancer Res.*, 1983, 43, 1295--
1300).

The compounds prepared by processes described
herein are antigens useful in adjuvant therapies as
vaccines capable of inducing antibodies immunoreactive
25 with epithelial carcinomas, for example, human colon,
lung and ovarian tumors. Such adjuvant therapies have
potential to reduce the rate of recurrence of cancer and
increase survival rates after surgery. Clinical trials
on 122 patients surgically treated for AJCC stage III
30 melanoma who were treated with vaccines prepared from
melanoma differentiation antigen GM2 (another tumor
antigen which like MBrl is a cell surface carbohydrate)

5 demonstrated in patients (lacking the antibody prior to immunization) a highly significant increase in disease-free interval (P. O. Livingston, et al., *J. Clin Oncol.*, 12, 1036 (1994)).

10 The effectiveness of a vaccine derived from a tumor-associated antigens increases with the greater specificity of the carbohydrate domain of the antigen. One such antigen is the glycolipid KH-1, immunocharacterized by Hakomori et al. who have proposed its structure as 1. (Nudelman, E.; Levery, S.B.; Kaizu, T; Hakomori, S.-I., *J. Biol. Chem.*, 1986, 261, 11247. 15 Kaizu, T.; Levery, S.B.; Nudelman, E; Stenkamp, R.E.; Hakomori, S.-I, *J. Biol. Chem.*, 1986, 261, 11254; Kim, S.Y.; Yuan, M.; Itzkowitz, S.H.: Sun, Q.; Kaizu, T.; Palekar, A; Trump, B.F.; Hakamori, S.-I, *Cancer Res.*, 20 1986, 46, 5985.)

This antigen has been claimed to be a highly specific marker for malignancy and pre-malignancies involving colonic adenocarcinoma. The nonasaccharide character of 1 (Figure 1) is unique from a structural 25 standpoint. The crystallographically derived presentation of the monoclonal antibody BR 96 bound to a Le^y tetrasaccharide glycoside has been reported. (Jeffery, P.D.; Bajorath, J.; Chang, C.Y.; Dale, Y.; Hellstrom, I.; Hellstrom, E.K.; Sheriff, S., *Nature Structural Biology*, 30 1995, 2, 456.) The structure of the BR96:Le^y complex suggested that this antibody might also have the capacity to recognize higher order fucosylated arrays.

5 Accordingly, the present invention relates to
the total synthesis not only of 1 itself, but of
congeners (cf. structure 2) which are suitable for
conjugation to appropriate bioactive carrier systems.

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Summary of the Invention

 Therefore, one object of the present invention
is to provide processes for the preparation of the KH-1
15 and N3 antigens, as well as related analogues thereof,
useful as anticancer therapeutics.

 Another object of the present invention is to
provide various compounds useful as intermediates in the
preparation of KH-1 and N3 and analogues thereof. A
20 further object of the present invention is to provide
methods of preparing such intermediates.

 An additional object of the invention is to
provide compositions comprising any of the analogues of
KH-1 and N3 available through the preparative methods of
the invention and pharmaceutical carriers useful in the
25 treatment of subjects suffering from cancer. A further
object of the invention is to provide methods of
treatment of cancer using any of the analogues of KH-1
and N3 alone or conjugated to suitable carriers as
disclosed herein available through the preparative
30 methods of the invention, optionally in combination with
pharmaceutical carriers.

Figure 1 show the structure of the cell surface antigen KH-1 ceramide and its bioconjugateable O-allyl ether form.

10

Figure 2 provides synthetic Scheme 1. Reagents: (a) (i) 3,3-dimethyldioxirane, CH_2Cl_2 ; (ii) 4 or 5, ZnCl_2 , THF 65% for 6 & 55% for 7; (b) (i) TESOTf, Et_3N , DMAP, CH_2Cl_2 , 92%, (ii) $\text{I}(\text{coll})_2\text{ClO}_4$, PhSO_2NH_2 , 4 Å molecular sieves, CH_2Cl_2 , > 90%; (iii) LHMDs, EtSH, DMF > 90%; (c) (i) Ac_2O , Et_3N , DMAP, CH_2Cl_2 , 95%; (ii) $\text{I}(\text{coll})_2\text{ClO}_4$, PhSO_2NH_2 , 4 Å molecular sieves, CH_2Cl_2 , > 90%; (iii) LHMDs, EtSH, DMF (iv) Ac_2O , Et_3N , DMAP, CH_2Cl_2 , 85%; (d) K_2CO_3 , MeOH 80%; (e) (i) MeOTf, di-*t*-butylpyridine, $\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$ (2:1), 4 Å MS (55%), (ii) K_2CO_3 , MeOH (85%); (f) (i) MeOTf, di-*t*-butylpyridine, $\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$ (2:1), 4 Å MS (60%); (ii) Ac_2O , Py, DMAP, CH_2Cl_2 (95%); (g) TBAF:AcOH (93%).

20

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Figure 3 provides synthetic Scheme 2. Reagents: (a) 14, $\text{Sn}(\text{OTf})_2$, Tol:THF(10:1), 4 Å MS (60%); (b) (i) 3,3-dimethyldioxirane, CH_2Cl_2 ; (ii) EtSH, CH_2Cl_2 , H^+ (cat); (iii) Ac_2O , Py, CH_2Cl_2 60% (3 steps) (c) 17, MeOTf, $\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$ (2:1), 4 Å MS (55%); (d) (i) Lindlar's catalyst, H_2 , palmitic anhydride, EtOAc, 85% (ii) Na, NH_3 , THF; (MeOH quench); (iii) Ac_2O , Et_3N , DMAP, CH_2Cl_2 (iv) MeONa, MeOH, 70% (3 steps); (e) (i) Na, NH_3 , THF; (MeOH quench); (ii) Ac_2O , Et_3N , DMAP, CH_2Cl_2 ; (iii) 3,3-

30

5 dimethyldioxirane, CH_2Cl_2 ; (iv) Allyl Alcohol (v) MeONa, MeOH, 60%.

Figure 4 provides a synthetic strategy for N3 antigen.

10 **Figure 5** provides a synthetic strategy for the Le x donor portion.

Figure 6 provides a synthetic strategy for the Le a donor portion.

15 **Figure 7** provides a synthetic strategy for the N3 acceptor portion.

Figure 8 provides a 2+2 coupling for the major N3 antigen.

Figure 9 provides a 2+4 and 1+1 coupling for the N3 antigen.

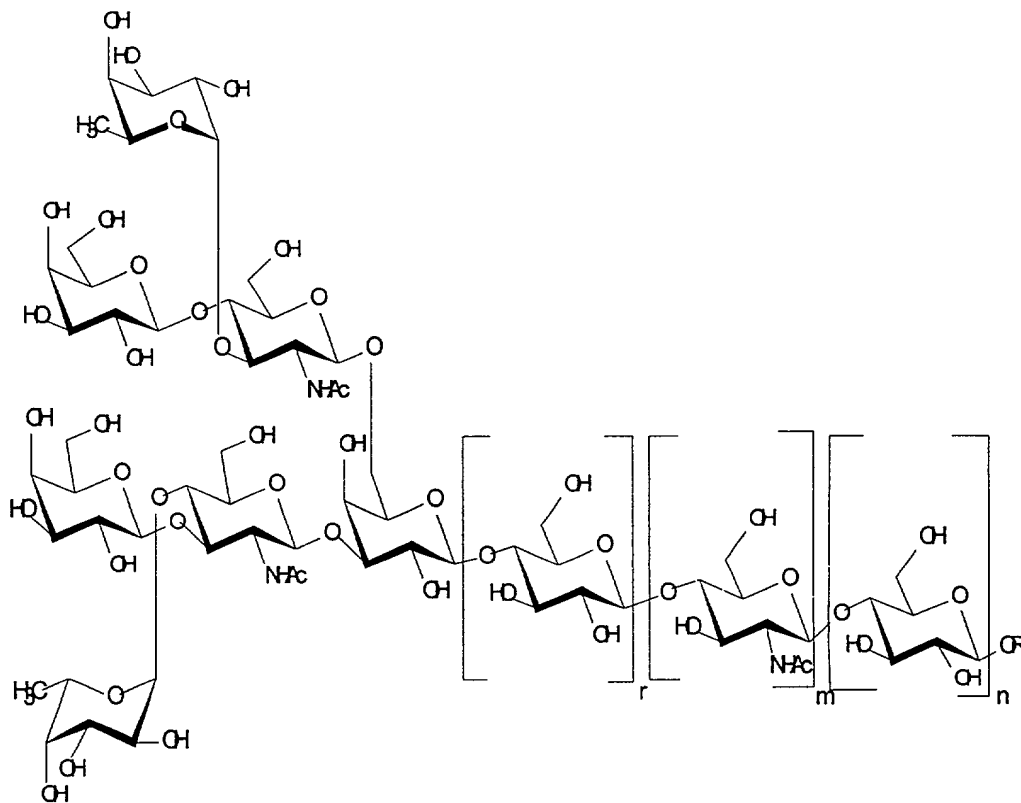
25 **Figure 10** provides a pathway for deprotection of the major N3 epitope.

Figure 11 provides a synthetic strategy for the KH-1 tetrasaccharide and hexasaccharide intermediates.

30 **Figure 12** illustrates the direct coupling of KH-1 to KLH.

Detailed Description of the Invention

The subject invention provides a compound having the structure:



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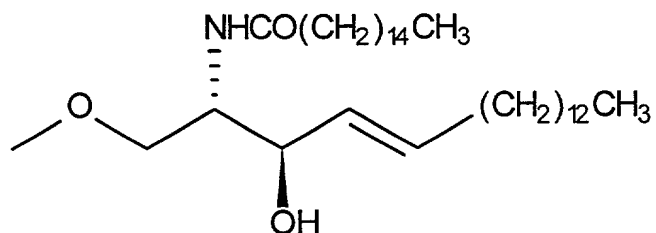
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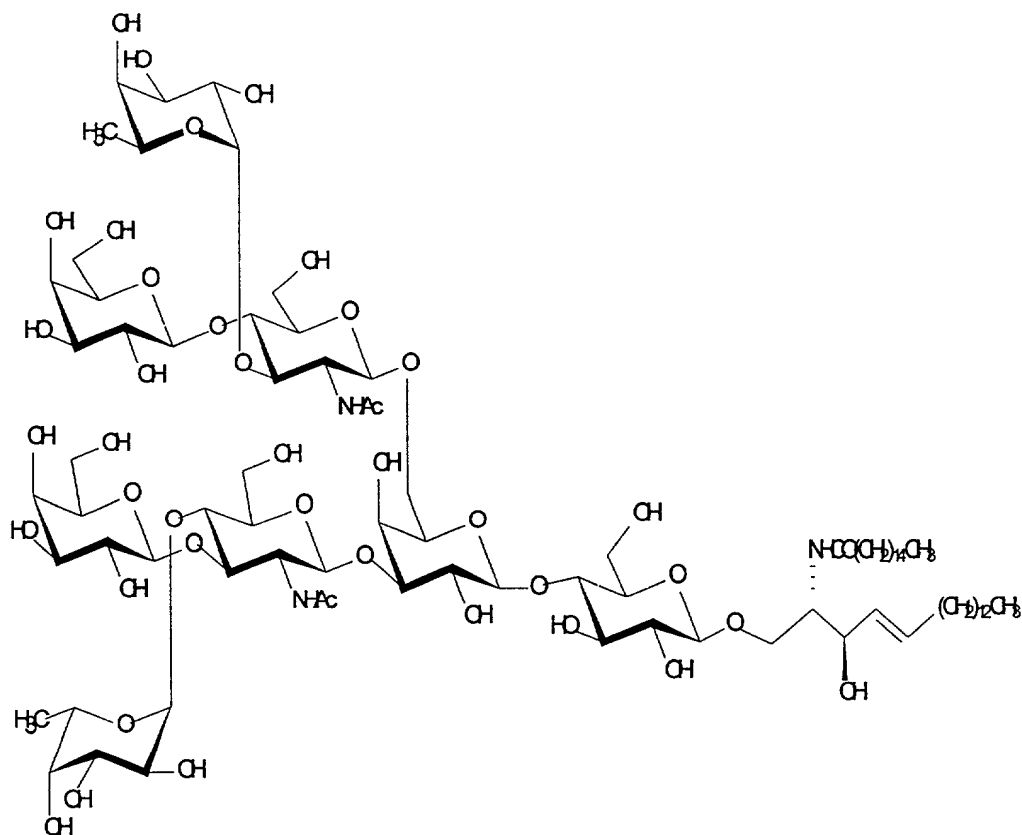
wherein R is H, substituted or unsubstituted alkyl, aryl or allyl, or an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, which amino acyl moiety or residue bears an ω -amino group or an ω -(C=O)- group, which group is linked to O via a polymethylene chain having the structure $-(CH_2)_s-$, where s is an integer between about 1 and about 9, or a moiety having the structure:



and wherein r, m and n are independently 0, 1, 2 or 3.

5

The present invention also provides a compound having the structure:



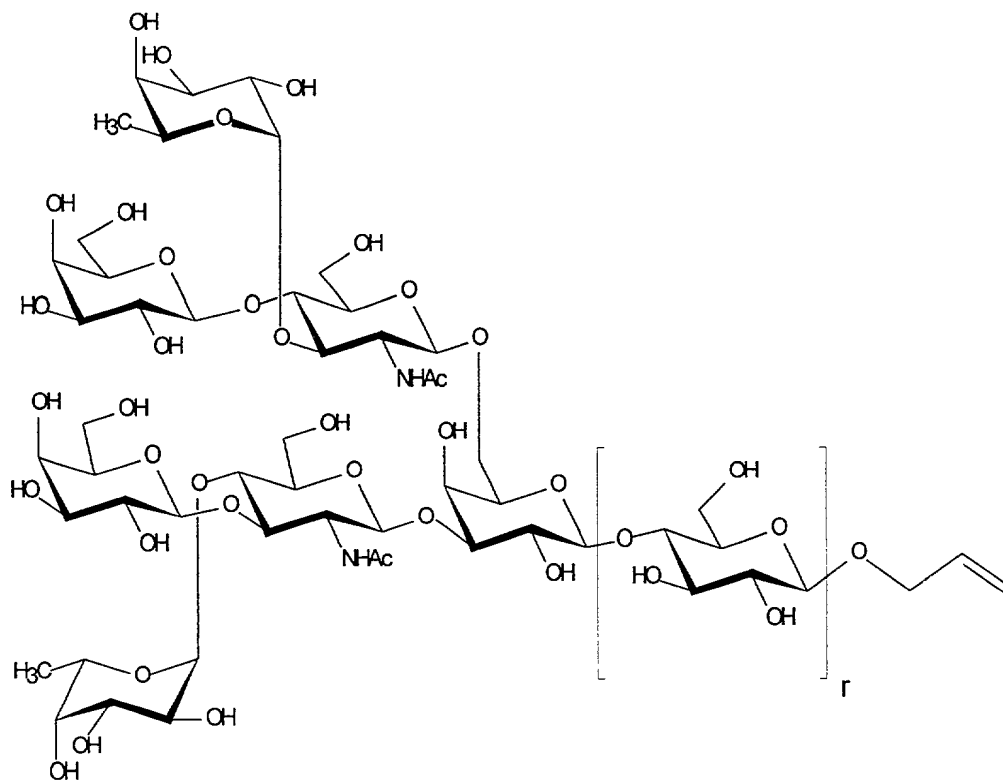
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5 In one embodiment, the invention provides a compound wherein the protein is bovine serum albumin or KLH.

The invention also provides a compound having the structure:

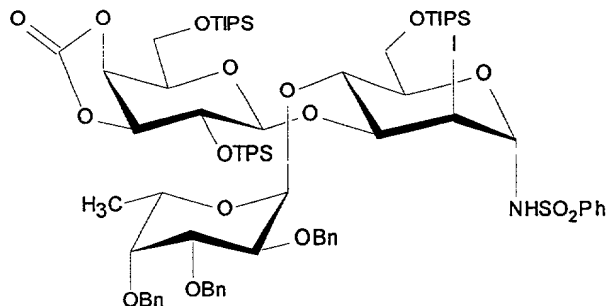
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5 wherein r is 0, 1, 2, 3 or 4. In one embodiment,
the invention provides the compound wherein r is 1.

The invention further provides a method of
preparing a trisaccharide iodosulfonamide having the
structure:

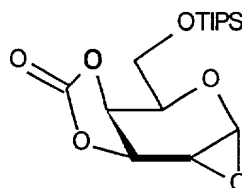
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which comprises:

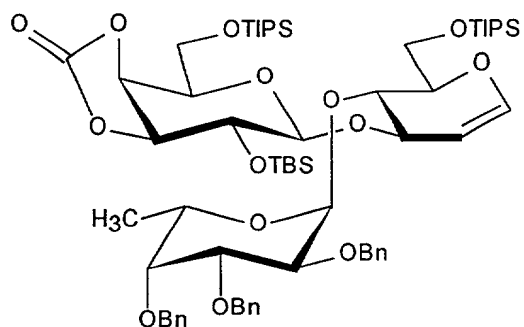
(a) (i) coupling a disaccharide glycal with an epoxide
having the structure:

15



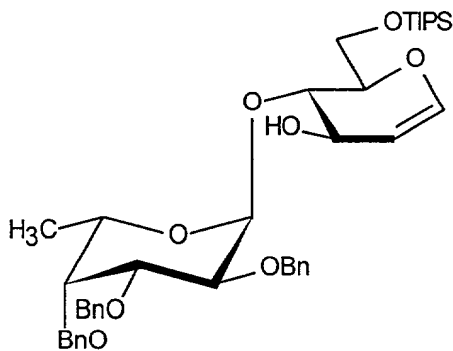
20 under suitable conditions to form a trisaccharide
intermediate; and

(ii) etherifying the trisaccharide intermediate with a
suitable protecting agent to form a trisaccharide glycal
having the structure:



and

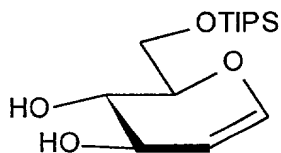
(b) reacting the trisaccharide glycal formed in step (c) with an iodosulfonamidating agent under suitable conditions to form the trisaccharide iodosulfonamide. In one embodiment, the invention provides the method wherein the disaccharide glycal has the structure:



is prepared by a process which comprises:

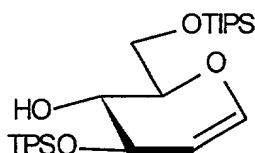
(a) protecting a glucal having the structure:

5



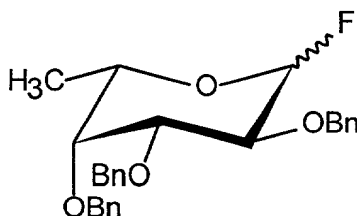
with a silylating agent under suitable conditions to form a protected glucal having the structure:

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(b) (i) alkylating the protected glucal formed in step (a) with a fucosylfluoride having the structure:

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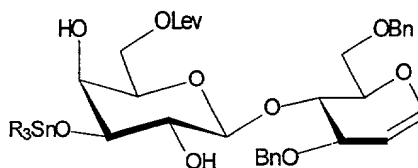
and

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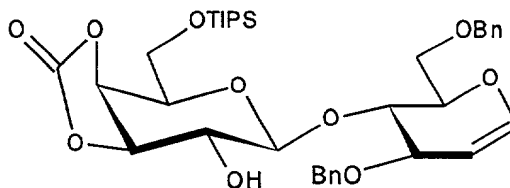
(ii) deprotecting under suitable conditions to form the disaccharide glycal. In one embodiment, the invention provides the method wherein the silylating agent in step (a) is triphenylsilyl chloride. In another embodiment, the invention provides the method wherein the alkylating step is effected in the presence of an ionizing salt, and the ionizing salt is AgClO_4 . In an additional embodiment, the invention provides the method wherein the conditions of the deprotecting step comprise a base. In yet another

5 embodiment, the invention provides the method wherein the
base is potassium carbonate. The method also encompasses
the embodiment wherein the conditions of the coupling
comprise an acid. The method further encompasses the
embodiment wherein the acid is a Lewis acid. One example
10 of the Lewis acid is zinc dichloride. One example of the
silylating agent used is TBSOTf. The iodosulfonamidating
agent of step (b) above may comprise $\text{I}(\text{coll})_2\text{ClO}_4$ and
 PhSO_2NH_2 .

The present invention also provides a method of
15 preparing a disaccharide stannane having the structure:



20 which comprises:
(a) (i) deprotecting a disaccharide glucal having the
structure:

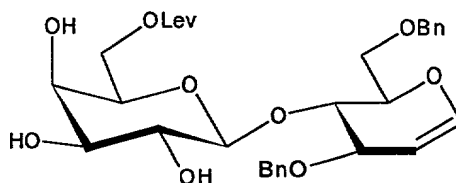


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10 under suitable conditions to form a deprotected intermediate; and

(ii) selectively reprotecting the deprotected intermediate with levulinic acid under suitable conditions to form a disaccharide levulinate having the structure:

15

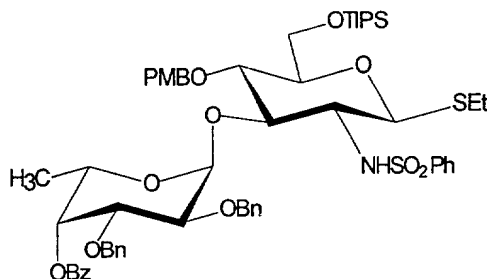


and

(b) reacting the disaccharide levulinate formed in step (a) with a distannyl oxide having the formula $(R_3Sn)_2O$, wherein R is linear or branched chain alkyl or aryl, under suitable conditions to form the disaccharide stannane. The invention encompasses the method wherein the conditions of the deprotecting step comprise a fluoride salt. The invention further encompasses the method wherein the fluoride salt is a tetraalkylammonium fluoride. The method additionally encompasses the method wherein the tetraalkylammonium fluoride salt is tetra-n-butylammonium fluoride. The invention also encompasses the method wherein the conditions of the reprotecting

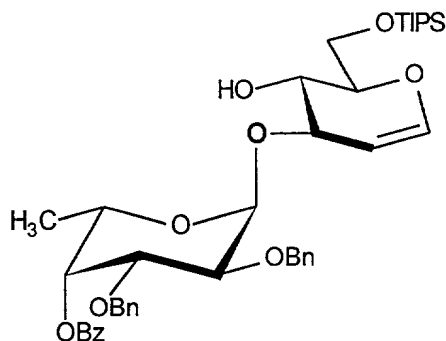
5 step comprise 2-chloro-1-methylpyridinium iodide. In one embodiment, the invention provides the method wherein R is n-Bu.

10 The present invention further provides a method of preparing a disaccharide ethylthioglycoside having the structure:



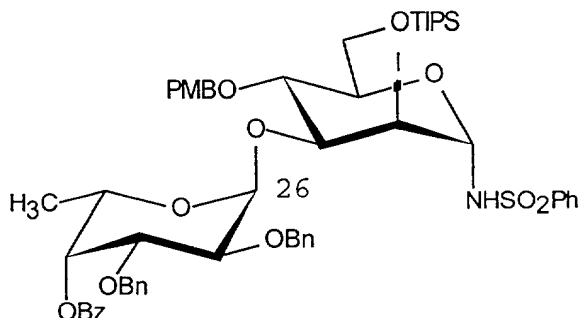
which comprises:

15 (a)(i) protecting a disaccharide glucal having the structure:



20 with a suitable protecting agent to form a protected disaccharide glucal; and

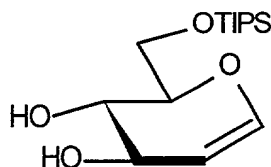
25 (ii) reacting the protected disaccharide glucal under suitable conditions with an iodosulfonamidating agent to form a disaccharide iodosulfonamide having the structure:



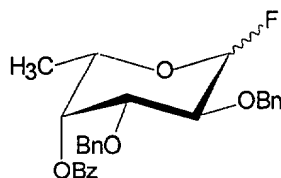
and

(b) treating the disaccharide iodosulfonamide formed in step (a)(ii) with ethanethiol under suitable conditions to form the disaccharide ethylthioglycoside. The method encompasses the embodiment wherein the disaccharide glucal is prepared by a process which comprises:

(a) alkylating a protected glucal having the structure:



with a fucosyl fluoride having the structure:

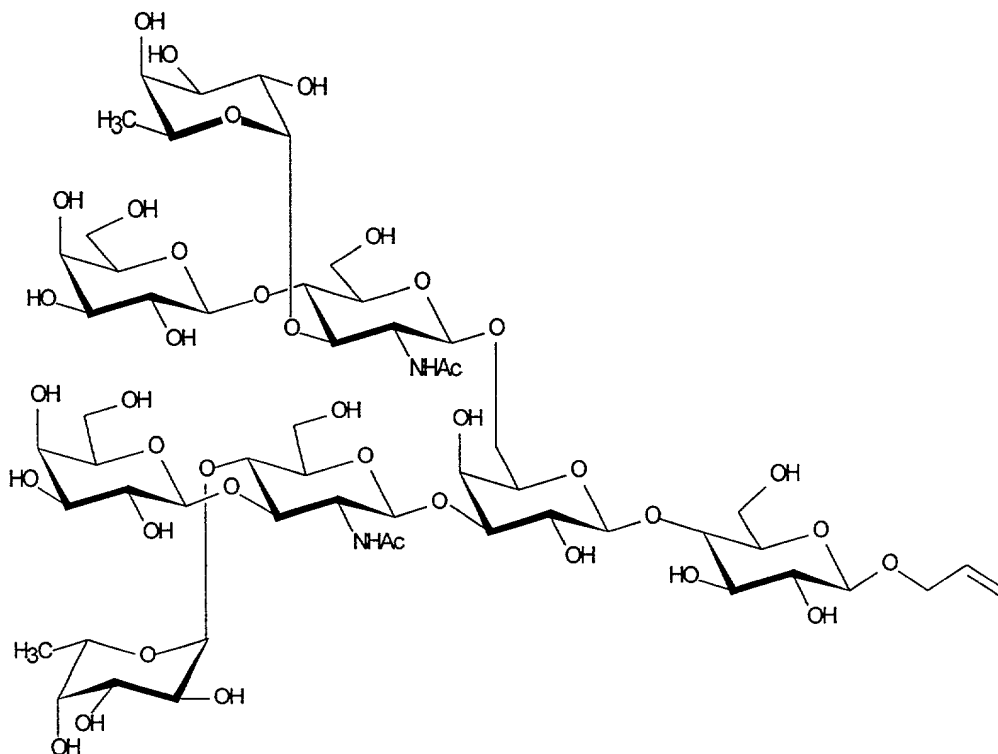


under suitable conditions to form the disaccharide glucal.

The method encompasses the embodiment wherein the conditions of the alkylating step comprise an ionizing salt. In addition, the method encompasses the example wherein the ionizing salt is AgClO_4 . The method also includes the example wherein the protecting agent is PMBCl . The method further encompasses the embodiment

5 wherein the iodosulfonamidating agent in step (b)(ii) comprises $I(coll)_2ClO_4$ and $PhSQNH_2$. The method also encompasses the embodiment wherein the conditions of the treating step comprise a base. The method also includes the instance wherein the base is LHMDS.

10 The invention also provides a method of preparing an N3 allyl glycoside having the structure:

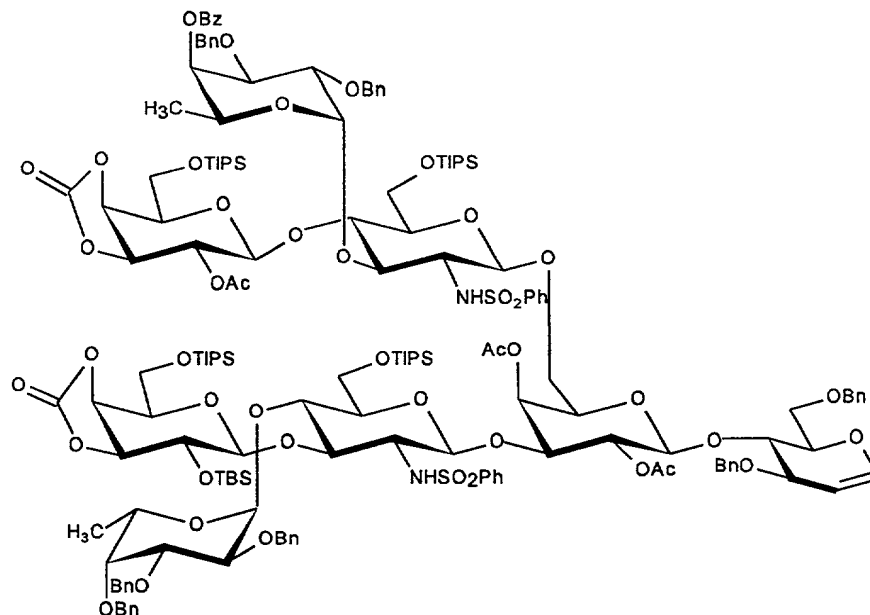


15 which comprises:

(a) desilylating a protected N3 glycal having the structure:

20

5



under suitable conditions to form a desilylated N3 glycal;

10 (b) deprotecting the desilylated N3 glycal formed in step (a) under suitable conditions to form a deprotected N3 glycal;

(c) treating the deprotected N3 glycal formed in step (b) with acetic anhydride in the presence of a suitable catalyst to form an N3 glycal acetate;

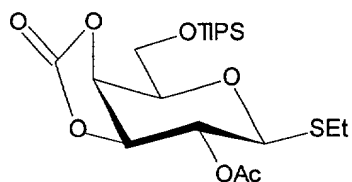
15 (d) epoxidizing the N3 glycal acetate formed in step (c) with an oxygen transfer agent under suitable conditions to form an N3 glycal epoxyacetate;

(e) cleaving the N3 glycal epoxyacetate formed in step (d) with allyl alcohol under suitable conditions to form an N3 glycal allyl ether; and

20 (f) saponifying the N3 glycal allyl ether under suitable conditions to form the N3 allyl glycoside.

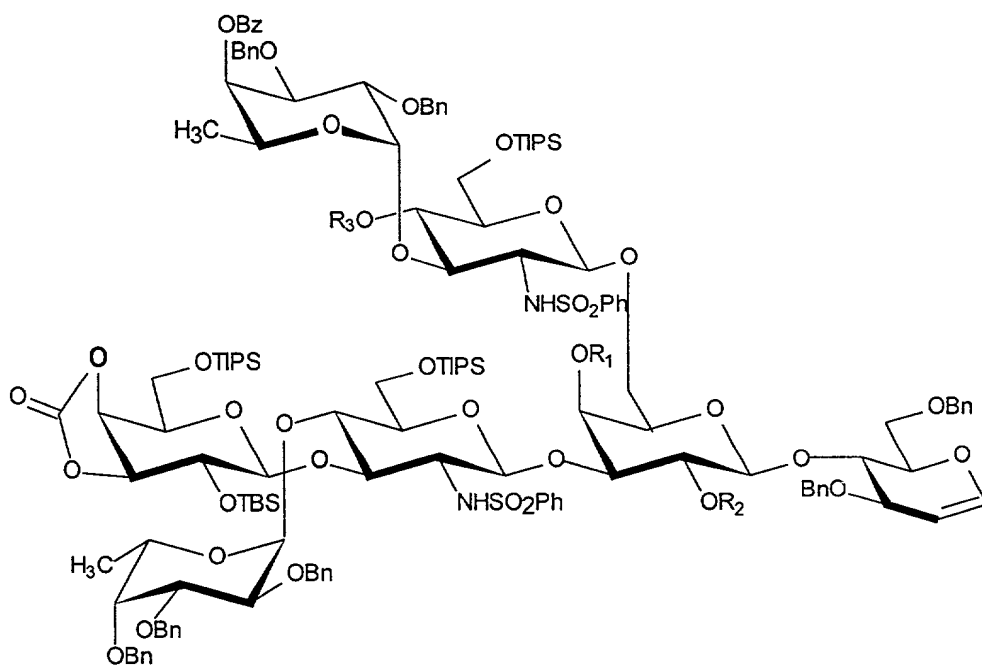
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The invention also encompasses the method wherein the protected N3 glycal is prepared by a process which comprises coupling an ethylthioglycoside having the structure:



10

heptasaccharide glycal having the structure:



15

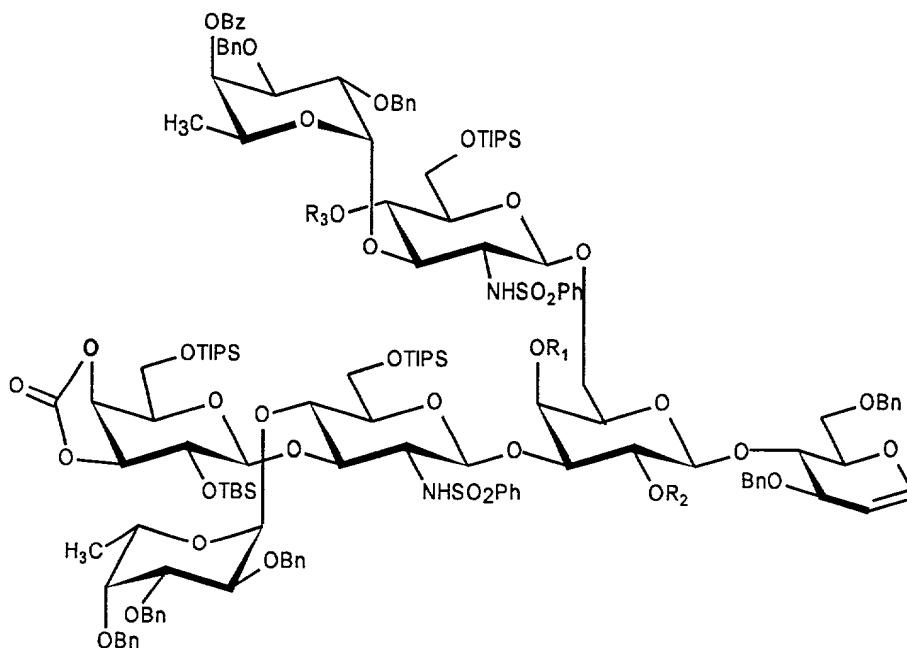
wherein R_1 and R_2 are Ac and R_3 is H, in the presence of an alkylating agent under suitable conditions to form the protected N3 glycal. The invention encompasses the method wherein the alkylating agent is MeOTf. The invention also encompasses the method wherein the conditions of the

20

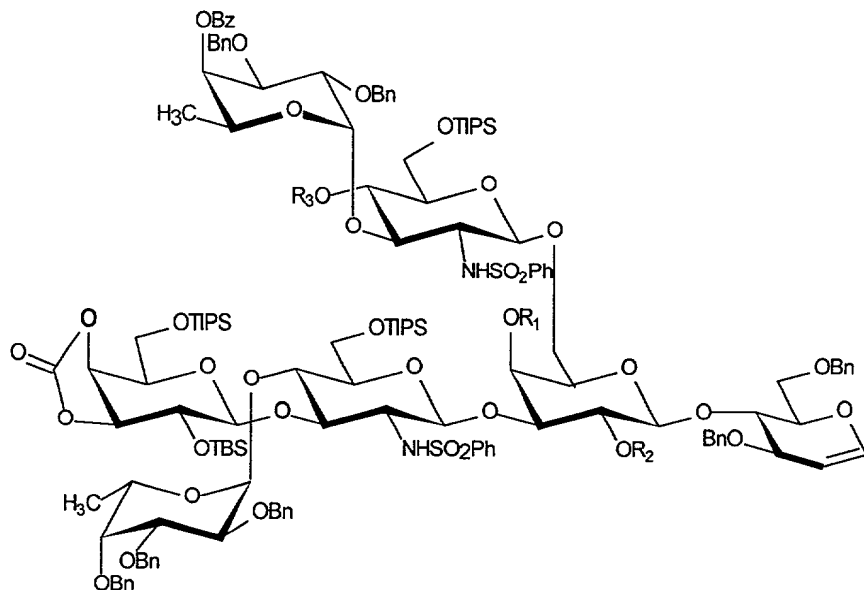
5 desilylating step comprise a fluoride salt. The invention also encompasses the method wherein the fluoride salt is a tetraalkylammonium fluoride. The invention also encompasses the method wherein the tetraalkylammonium fluoride is tetra-n-butylammonium fluoride.

10 The invention further includes the method wherein the catalyst in the treating step is 2-N,N-dimethylaminopyridine. The invention also encompasses the method wherein the oxygen transfer agent is 3,3-dimethyldioxirane.

15 The present invention encompasses a method of preparing a heptasaccharide glycal diacetate intermediate having the structure:



5 wherein R_1 and R_2 are Ac and R_3 is H, which comprises:
 (a) (i) monoacylating a heptasaccharide glycal having the structure:

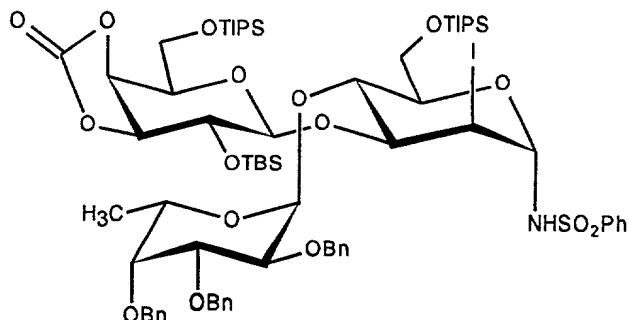


10

wherein R_1 and R_2 are H and R_3 is PMB; with acyl anhydride in the presence of a catalyst under suitable conditions to form a heptasaccharide glycal monoacetate;
 15 (ii) treating the heptasaccharide glycal monoacetate formed in step (a)(i) with an acyl anhydride in the presence of a catalyst under conditions suitable to form a heptasaccharide glycal diacetate;
 (iii) deprotecting the heptasaccharide glycal diacetate
 20 under suitable conditions to form the heptasaccharide glycal diacetate intermediate.

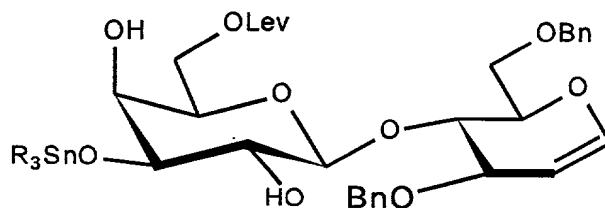
The invention encompasses the method wherein the heptasaccharide glycal is prepared by a process which comprises:

5 (a)(i) reacting a trisaccharide iodosulfonamide having the structure:



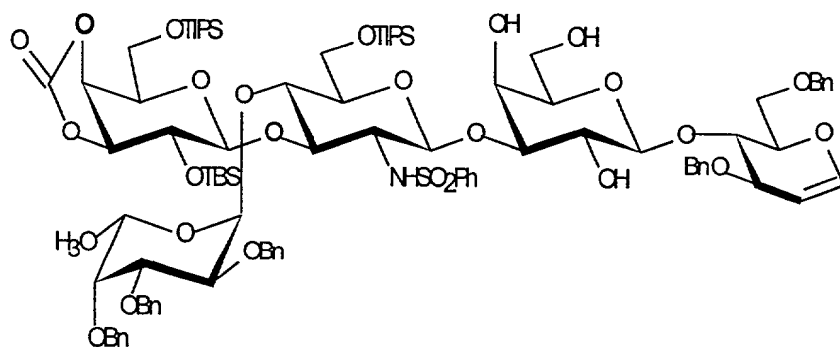
with a disaccharide stannane having the structure:

10



under suitable conditions; and (ii) deprotecting under suitable conditions to form a pentasaccharide glycal having the structure:

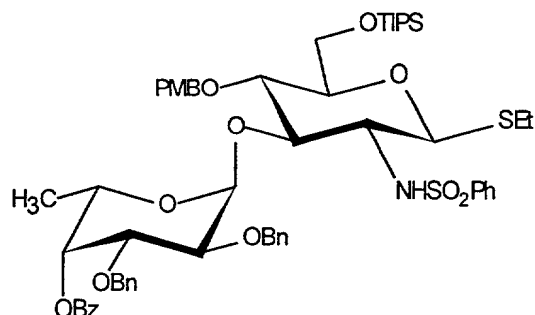
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and

(b) coupling the pentasaccharide glycal formed in step
20 (a) with an ethylthioglycoside having the structure:

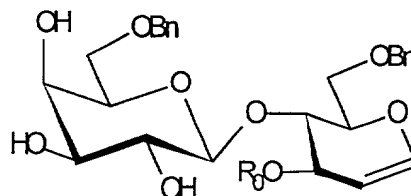
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under suitable conditions to form the heptasaccharide glycal. The invention encompasses the method wherein the conditions of the reacting step comprise an ionizing agent.

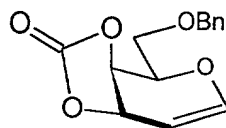
The invention also encompasses the method wherein the ionizing agent is AgBF_4 .

The invention further encompasses a method of preparing a protected disaccharide having the structure:



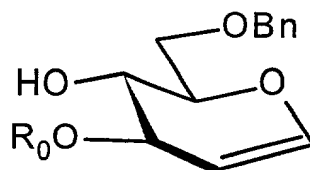
wherein R_0 is a linear or branched chain alkyl, arylalkyl, trialkylsilyl, aryldialkylsilyl, diarylalkylsilyl, and triarylsilyl, which comprises:

(a) (i) epoxidizing a galactal carbonate having the structure:



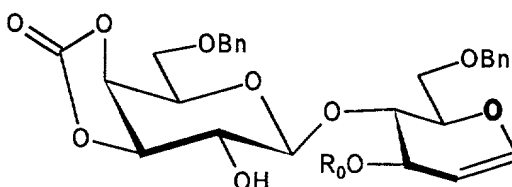
5 with an oxygen transfer agent under suitable conditions to form an epoxide galactal; and
 (ii) coupling the epoxide galactal formed in step (a) (i) with a doubly protected galactal having the structure:

10



under suitable conditions to form a disaccharide carbonate having the structure:

15



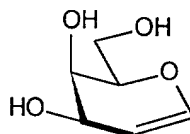
and (b) saponifying the disaccharide carbonate formed in step (a) (ii) under suitable conditions to form the protected disaccharide.

20

The invention encompasses the method wherein the galactal carbonate is prepared by a process which comprises:

(a) protecting a galactal having the structure:

25

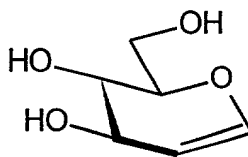


5 with an alkylating agent under suitable conditions to form a first protected galactal; and

(b) treating the first protected galactal formed in step (a) with a carbonate-forming reagent under conditions
10 suitable to form the galactal carbonate. The invention further provides the method wherein the carbonate-forming reagent is $(\text{Im})_2\text{CO}/\text{DMAP}$.

The invention also provides a method wherein the doubly protected galactal is prepared by a process
15 which comprises:

(a) protecting a second galactal having the structure:

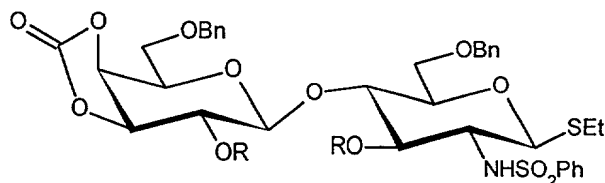


20 with an alkylating agent under conditions suitable to form a second protected galactal; and

(b) protecting the second protected galactal formed in step (a) with an alkylating agent which may be the same or different from that of step (a) under conditions
25 suitable to form the doubly protected galactal. The invention encompasses the method wherein each alkylating agent is independently an alkyl, arylalkyl, trialkylsilyl, aryldialkylsilyl, diarylalkylsilyl or triarylsilyl halide or triflate. The invention further
30 encompasses the method wherein the alkylating agent is benzyl bromide. In one example, the alkylating agent is TES-Cl. The method also encompasses the method wherein

5 the oxygen transfer agent is DMDO. The method further encompasses conditions for the coupling step comprising ZnCl_2 in THF. The additionally encompasses conditions for the saponifying step comprising K_2CO_3 in methanol.

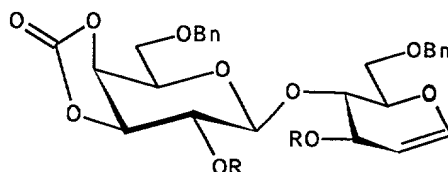
10 The present invention further provides a method of preparing an ethylthioglycoside having the structure:



15 wherein R is C_{1-9} linear or branched chain alkyl, arylalkyl, trialkylsilyl, aryldialkylsilyl, diarylalkylsilyl, and triarylsilyl, which comprises:

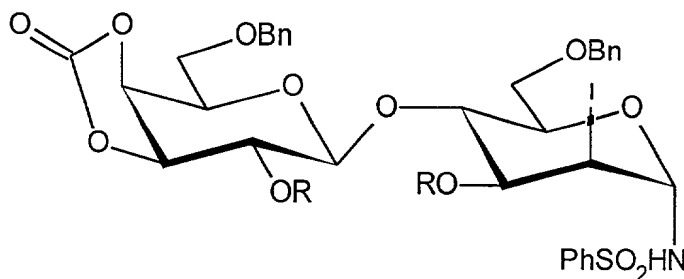
(a) treating a protected disaccharide carbonate having the structure:

20



with an iodosulfonamidating agent under suitable conditions to form a disaccharide iodosulfonamidate having the structure:

25

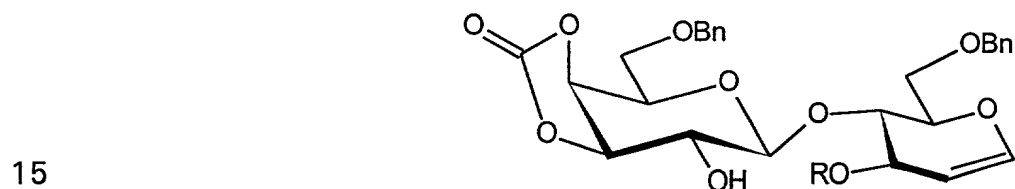


30

and

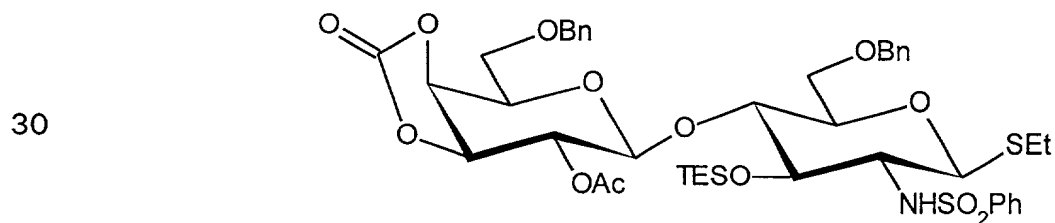
5 (b) reacting the disaccharide iodosulfonamidate formed in
step (a) with ethylthiol under suitable conditions to
form the ethylthioglycoside.

The invention also provides a method wherein
the protected disaccharide carbonate is prepared by a
10 process which comprises alkylating a disaccharide
carbonate having the structure:



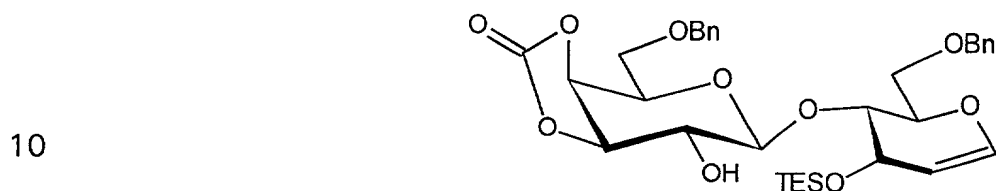
with an alkylating agent under suitable conditions to
form the protected disaccharide carbonate. The method
encompasses within the scope of the method any alkylating
agent selected from the group including an alkyl,
20 arylalkyl, trialkylsilyl, aryldialkylsilyl,
diarylalkylsilyl or triarylsilyl halide or triflate. An
example of the alkylating agent is TES-Cl. An example of
the the iodosulfonamidating agent is $I(coll)_2ClO_4$ and
 $PhSO_2NH_2$.

25 The present invention provides a method of
preparing an ethylthioglycoside having the structure:

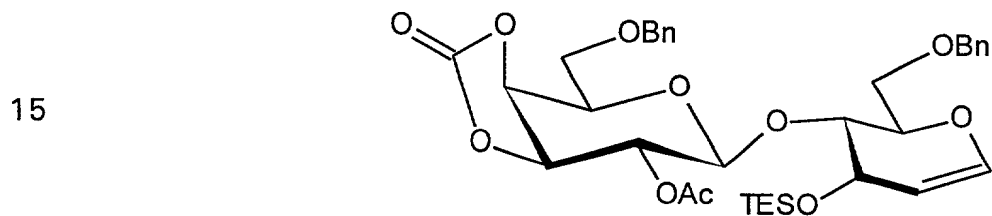


which comprises:

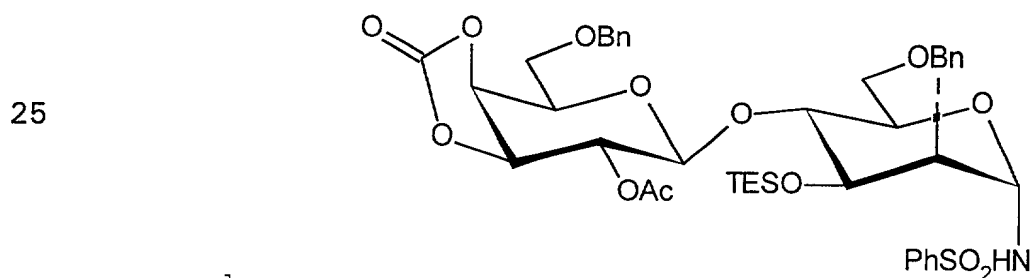
5 (a) acylating a disaccharide carbonate having the structure:



under suitable conditions to form an acylated disaccharide carbonate having the structure:



20 (b) treating the acylated disaccharide carbonate formed in step (a) with an iodosulfonamidating agent under suitable conditions to form a disaccharide iodosulfonamidate having the structure:



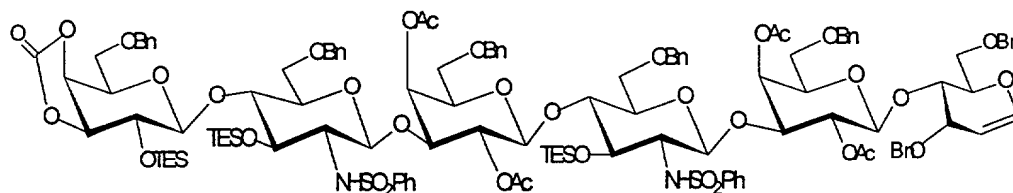
and

30 (c) reacting the iodosulfonamidate formed in the step (b) with ethyl thiol under suitable conditions to form the ethylthioglycoside. The invention encompasses the method wherein the conditions of the acylating step comprise

5 acetic anhydride/pyridine. An example of the iodosulfonamidating agent is $I(coll)_2ClO_4$ and $PhSO_2NH_2$.

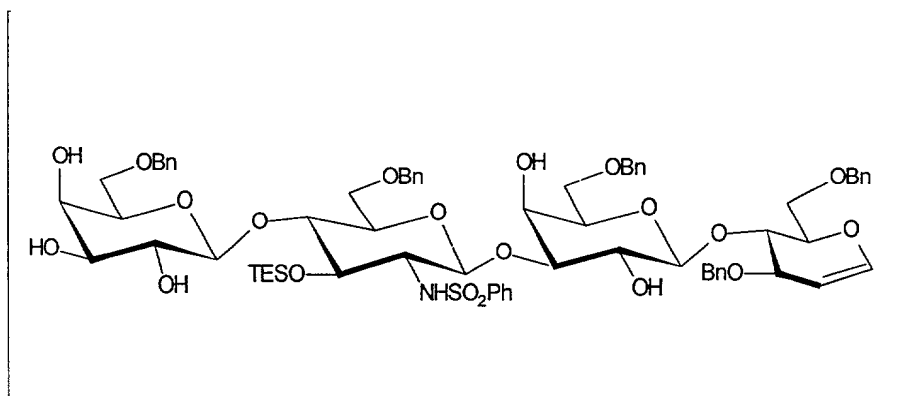
The present invention also provides a method of preparing a protected hexasaccharide having the structure:

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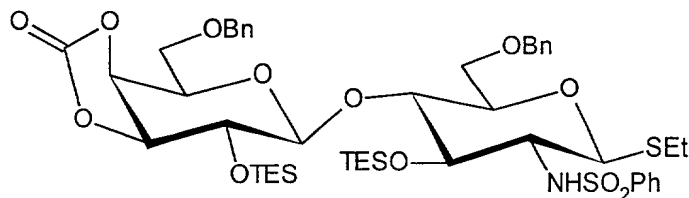
which comprises:

(a) reacting a protected tetrasaccharide having the structure:



15

with an ethylglycoside having the structure:

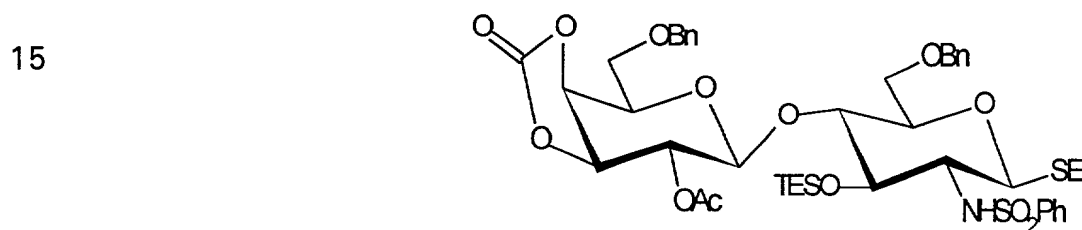


5 under suitable conditions to form a hexasaccharide intermediate; and

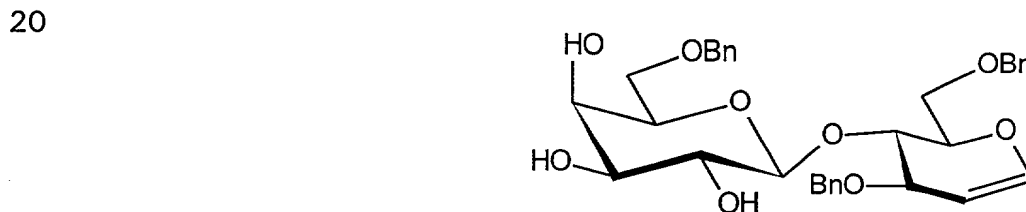
(b) acetylating the hexasaccharide intermediate formed in step (a) under suitable conditions to form the protected hexasaccharide.

10 The invention provides a method wherein the protected tetrasaccharide is prepared by a process which comprises:

(a) coupling an ethythioglycoside having the structure:



with a protected disaccharide having the structure:



25 under suitable conditions to form a protected tetrasaccharide carbonate; and

(b) saponifying the protected tetrasaccharide carbonate formed in step (a) under suitable conditions to form the protected tetrasaccharide. The invention encompasses the method wherein the conditions of the coupling step comprise MeOTf/MS. The invention also encompasses the method wherein the conditions of the saponifying step comprise K_2CO_3 in methanol.

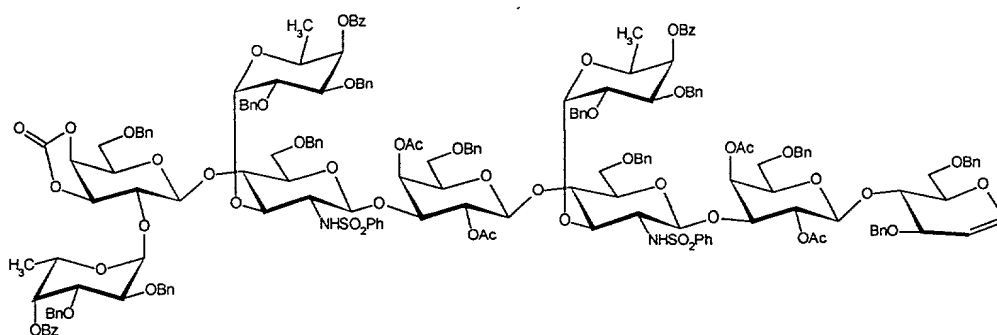
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The present invention provides a method of preparing a protected nonasaccharide having the structure:

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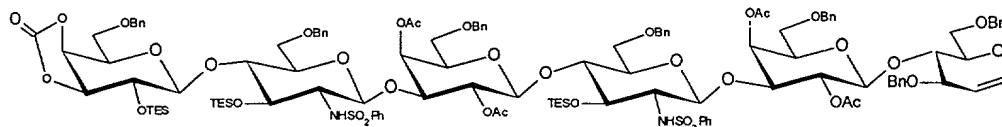


which comprises:

20

(a) deprotecting a protected hexasaccharide having the structure:

25

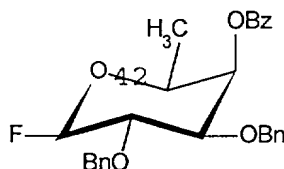


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under suitable conditions to form a partially deprotected hexasaccharide; and

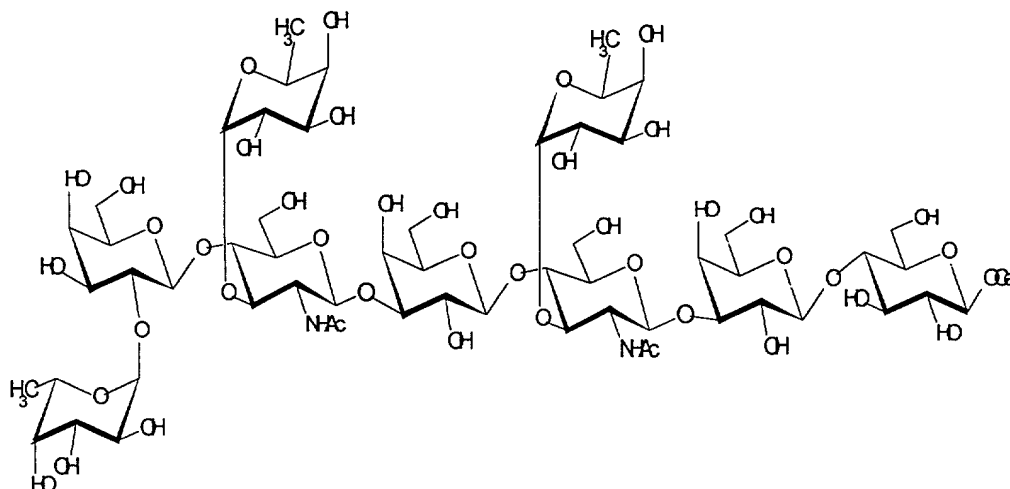
(b) coupling the partially deprotected hexasaccharide formed in step (a) with a fucosylfluoride having the structure:

35



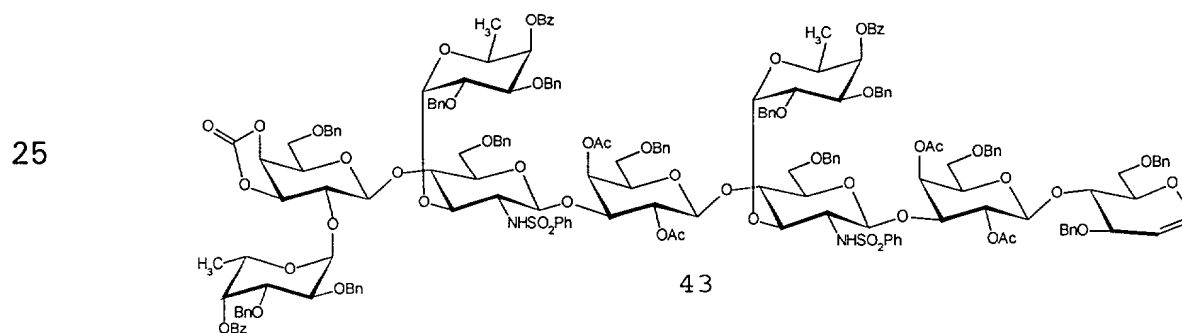
5 in the presence of an organometallic reagent under
 suitable conditions to form the protected nonasaccharide.
 The method encompasses conditions of the deprotecting
 step comprising a fluoride salt. The fluoride salt may
 be a tetraalkylammonium fluoride. Specifically, the
 10 fluoride salt may be TBAF. The invention encompasses the
 method wherein the organometallic reagent is
 $\text{Sn}(\text{OTf})_2/\text{DTBP}$.

The present invention also provides a method of
 preparing a protected nonasaccharide ceramide having the
 15 structure:



which comprises:

20 (a) epoxidizing a protected nonasaccharide having the
 structure:



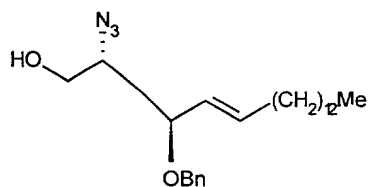
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with an oxygen transfer agent under suitable conditions to form a protected nonasaccharide epoxide;

(b) coupling the protected nonasaccharide epoxide formed in step (a) with an azide having the structure:

15



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under suitable conditions to form a nonasaccharide azide intermediate;

(c) reductively acylating the azide intermediate with palmitic anhydride under suitable conditions to form a protected nonasaccharide ceramide;

25

(d) reducing the protected nonasaccharide ceramide formed in step (c) under suitable conditions to form a deprotected nonasaccharide ceramide;

(e) acylating the deprotected nonasaccharide ceramide under suitable conditions to form an acylated nonasaccharide ceramide; and

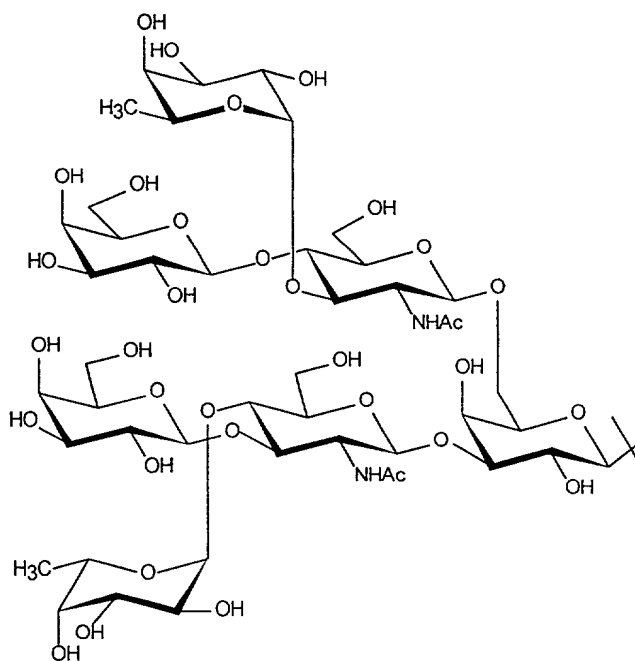
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(f) saponifying the acylated nonasaccharide ceramide under suitable conditions to form the nonasaccharide ceramide. The invention encompasses the method wherein

5 the oxygen transfer agent is DMDO. The invention also encompasses the method wherein the conditions of the coupling step comprise ZnCl_2 . The method further encompasses use of an azide intermediate which is reductively acylated in step (c) in the presence of
10 Lindlar's catalyst. The invention further encompasses the method wherein conditions of the saponifying step comprise MeONa in methanol.

The present invention provides a method of inducing antibodies in a subject, wherein the antibodies are capable of specifically binding with epithelial tumor cells cells, which comprises administering to the subject an amount of a compound which contains a determinant having a structure selected from the group consisting of:

(a)

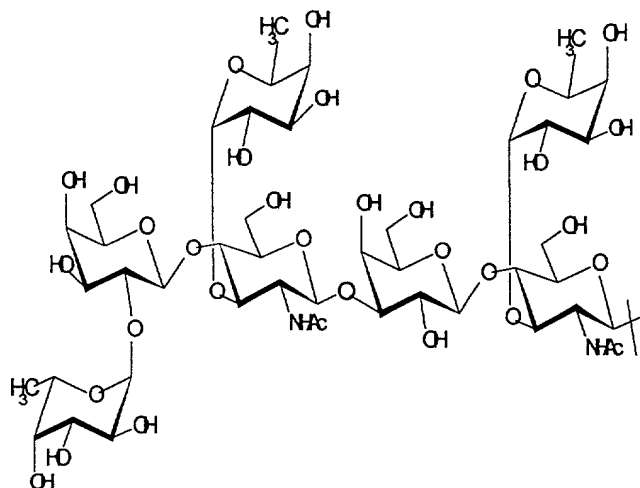


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and

10

(b)



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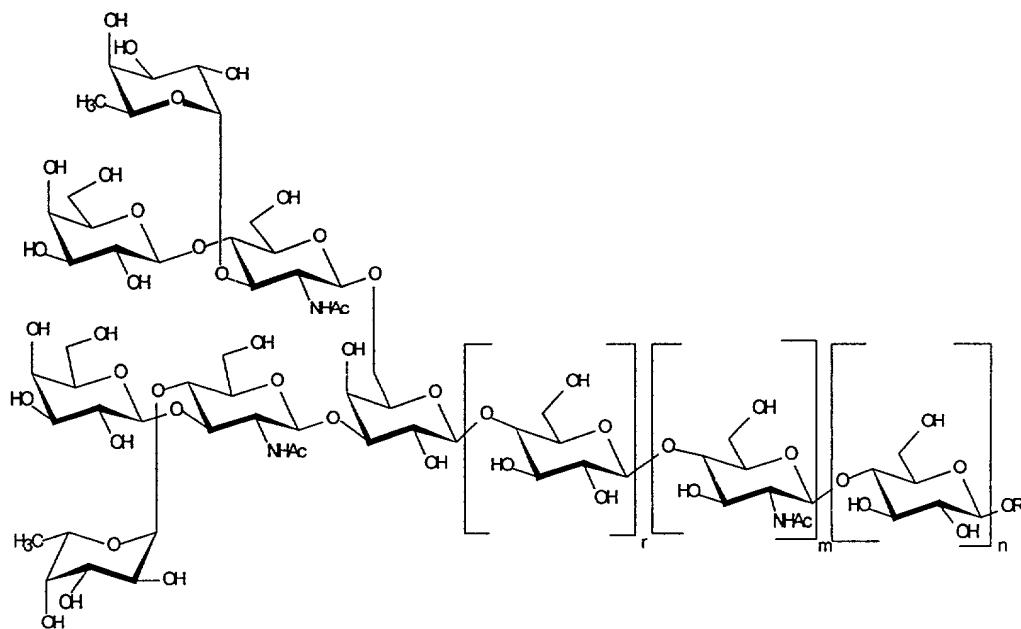
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which amount is effective to induce antibodies. In one embodiment, the invention encompasses a method wherein the compound is bound to a suitable carrier protein, said compound being bound either directly or by a cross-linker selected from the group consisting of a succinimide and an M_2 linker. Preferably, the compound contains a KH-1 or N3 epitope. The method specifically encompasses use of the carrier protein selected from the group consisting of bovine serum albumin, polylysine or KLH. The method also encompasses the method disclosed which further comprises co-administering an immunological adjuvant. In

5 particular, the adjuvant may include bacteria or liposomes. Specifically, the adjuvant may be *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21. In a certain embodiment, the method includes use of the compound having the structure:

10

(a)



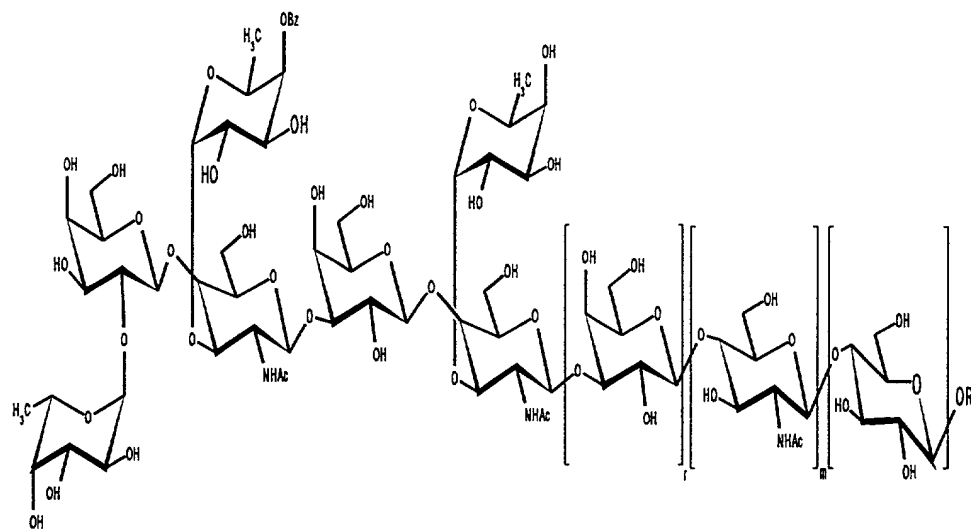
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or

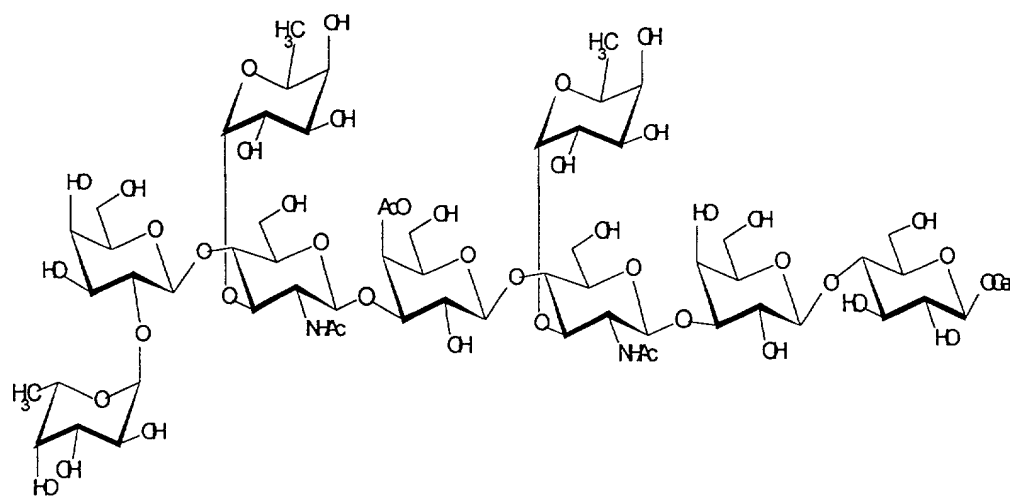
(b)

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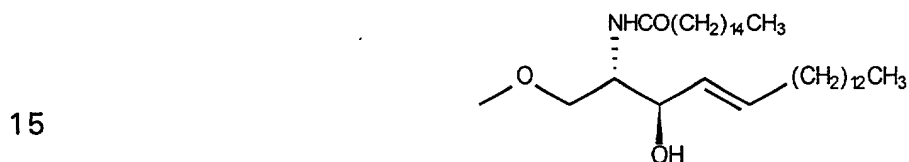
or

(c)



10

5 wherein R is H, substituted or unsubstituted alkyl, aryl
 or allyl, or an amino acyl moiety, an amino acyl residue
 of a peptide, an amino acyl residue of a protein, which
 amino acyl moiety or residue bears an ω -amino group or an
 ω -(C=O)- group, which group is linked to O via a
 10 polymethylene chain having the structure $-(CH_2)_s-$, where
 s is an integer between about 1 and about 9, or a moiety
 having the structure:



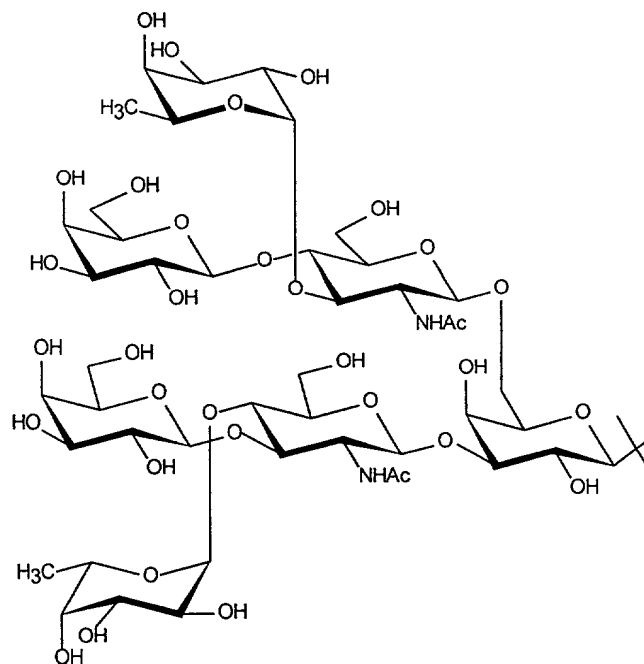
and wherein r, m and n are independently 0, 1, 2 or 3.
 The invention encompasses the method wherein the subject
 is in clinical remission or, where the subject has been
 treated by surgery, has limited unresected disease.

20 In the practice of the invention, the method
 encompasses the induction of antibodies capable of
 specifically binding with gastrointestinal tumor cells,
 colon tumor cells, lung tumor cells, prostate tumor
 cells.

25 In addition, the invention provides a method of
 treating a subject suffering from an epithelial cell
 cancer, which comprises administering to the subject an
 amount of a compound which contains a determinant having
 a structure selected from the group consisting of:

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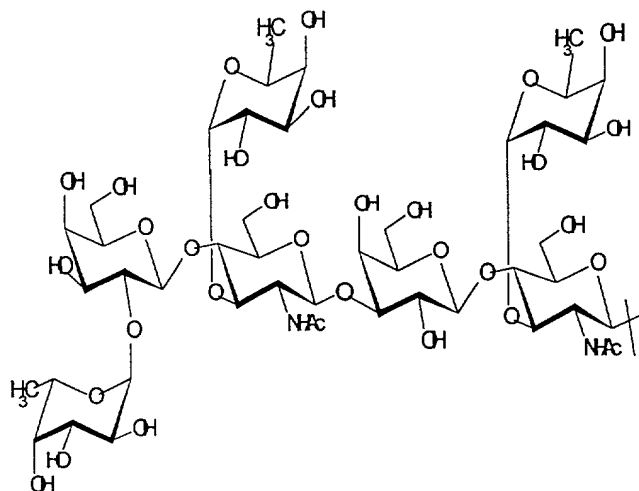
(a)



and

(b)

10

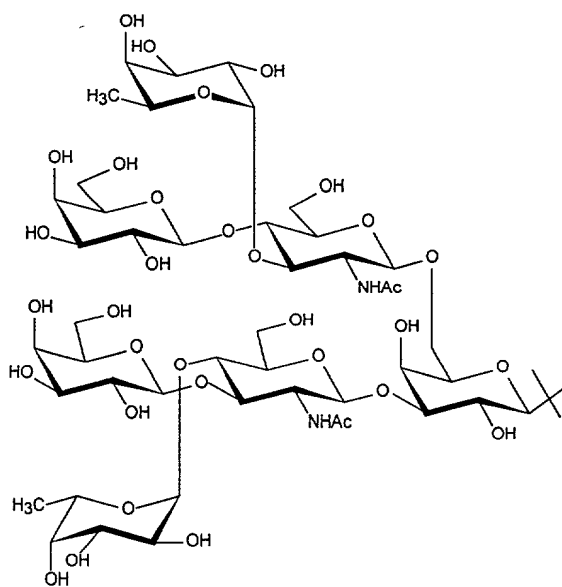


which amount is effective to treat the cancer. The method may be practiced wherein the compound is bound to a suitable carrier protein, said compound being bound

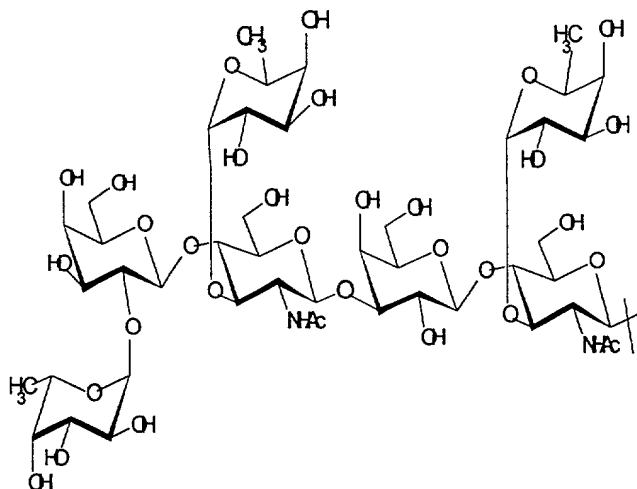
5 either directly or by a cross-linker selected from the
group consisting of a succinimide and an M₂ linker.
Favorably, the carrier protein is bovine serum albumin,
polylysine or KLH, and the compound contains a KH-1 or N3
10 epitope. The method may further comprise co-
administering an immunological adjuvant. The adjuvant is
bacteria or liposomes. In particular, the adjuvant is
Salmonella minnesota cells, bacille Calmette-Guerin or
QS21.

15 The invention further provides a method of
preventing recurrence of epithelial cell cancer in a
subject which comprises vaccinating the subject with a
compound which contains a determinant having the
structure:

(a)



and (b)

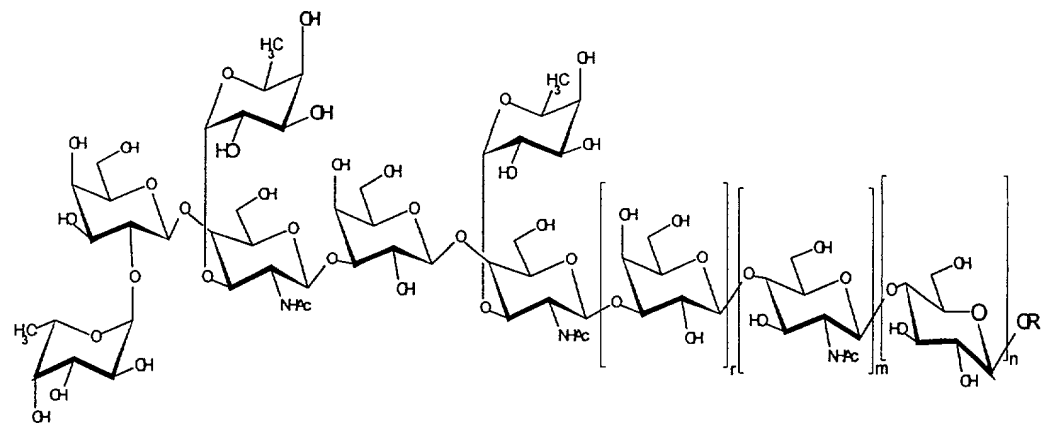


which amount is effective to induce the antibodies. The invention encompasses the method wherein the compound is bound to a suitable carrier protein. The method specifically encompasses use of any effective carrier protein including bovine serum albumin, polylysine or KLH. In addition, the method may further comprises co-administering an immunological adjuvant. The adjuvant may be bacteria or liposomes. In particular, the adjuvant may be *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21.

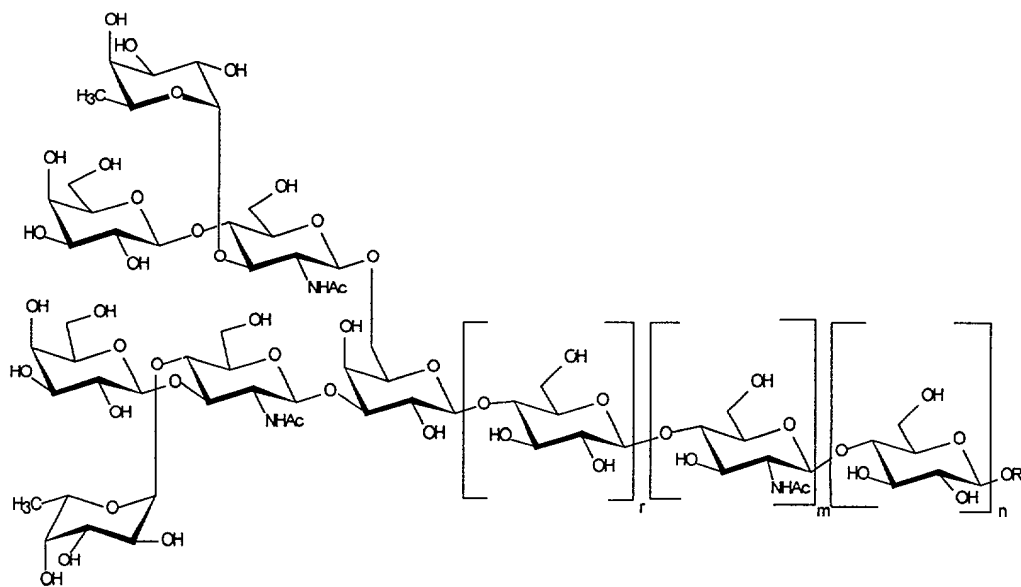
The method may carried out using a compound selected from the group consisting of:

(a)

5



and (b)

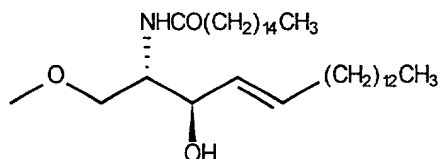


10

wherein R is H, substituted or unsubstituted alkyl, aryl or allyl, or an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, which amino acyl moiety or residue bears an ω -amino group or an ω -(C=O)- group, which group is linked to O via a polymethylene chain having the structure $-(CH_2)_s-$, where

15

5 s is an integer between about 1 and about 9, or a moiety having the structure:



10

and wherein r, m and n are independently 0, 1, 2 or 3.

The processes of the present invention for preparing KH-1 and N3 antigens and analogues thereof and intermediates thereto encompass the use of various alternate protecting groups known in the art. Those protecting groups used in the disclosure including the Examples below are merely illustrative. One of ordinary skill would understand how to substitute equivalent protecting groups for those illustrated.

20 The subject invention also provides pharmaceutical compositions for treating cancer comprising any of the analogues of KH-1 or N3 antigens as disclosed herein, optionally in combination with a pharmaceutically suitable carrier.

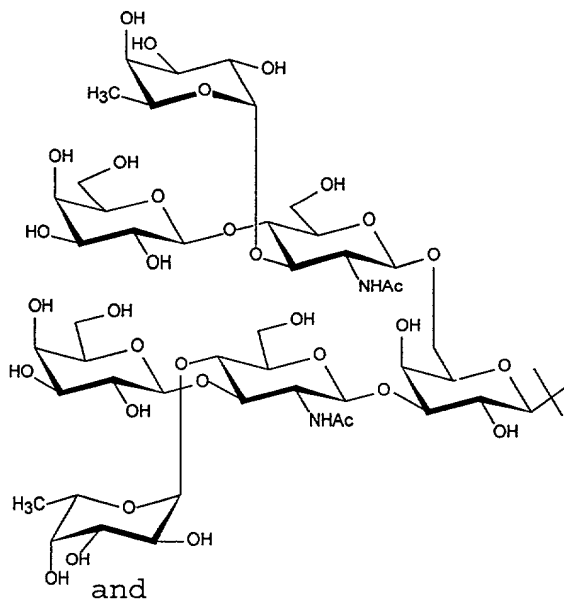
25 The subject invention further provides a method of treating cancer in a subject suffering therefrom comprising administering to the subject a therapeutically effective amount of any of the analogues of KH-1 or N3 antigens disclosed herein and a pharmaceutically suitable carrier.

30

5 The invention provides a method of preventing recurrence of an epithelial cell cancer in a subject which comprises vaccinating the subject with a compound which contains a determinant having the structure:

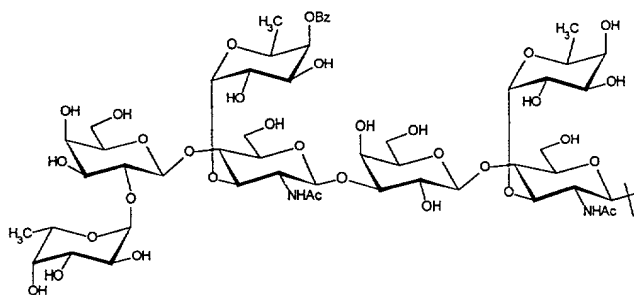
(a)

10



(b)

15



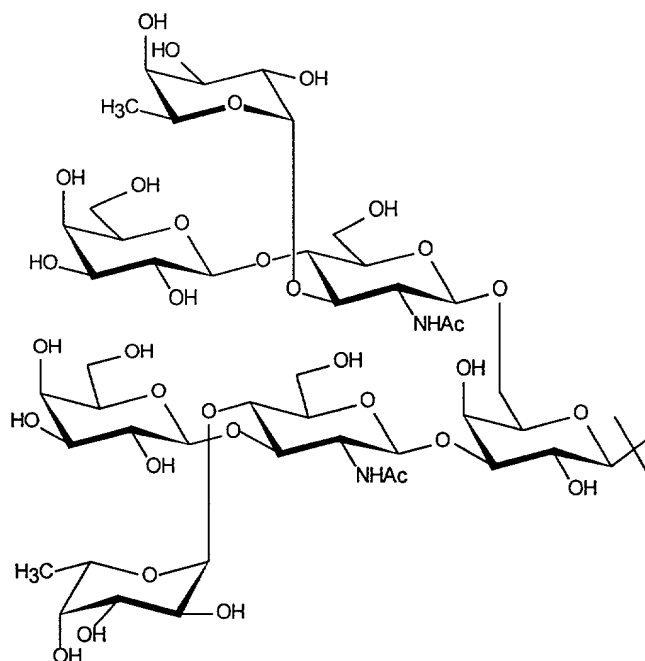
20

which amount is effective to prevent recurrence of an epithelial cell cancer.

The invention provides a composition comprising a compound which contains a determinant having a structure selected from the group consisting of:

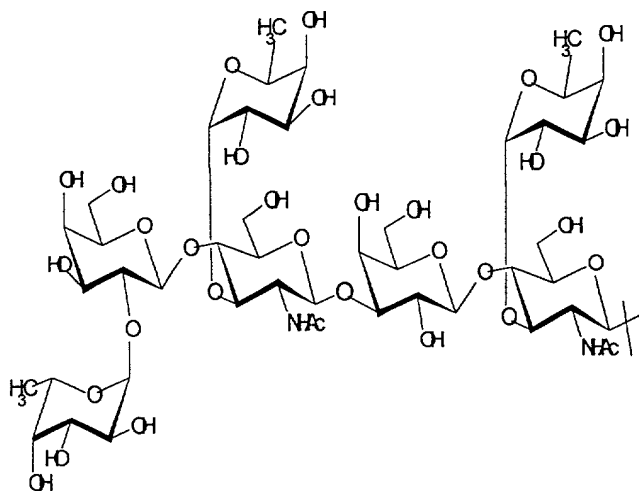
5

(a)



and

(b)



and optionally an immunological adjuvant and/or a pharmaceutically acceptable carrier.

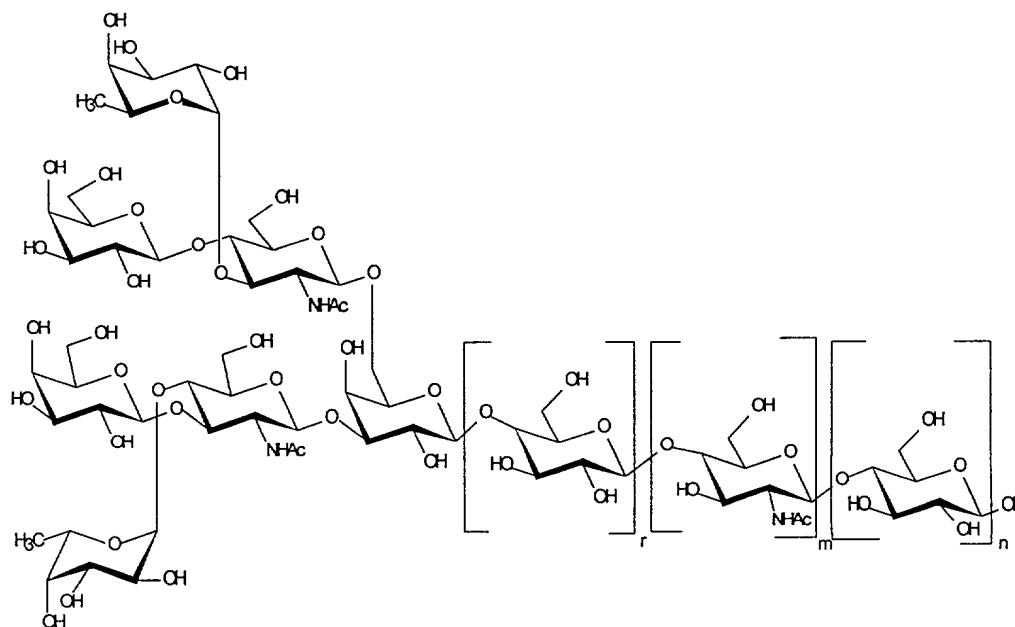
5 The invention also provides the composition wherein
the compound is bound to a suitable carrier protein, said
compound being bound either directly or by a cross-linker
selected from the group consisting of a succinimide and
an M_2 linker. The composition is also provided wherein
10 the carrier protein is bovine serum albumin, polylysine
or KLH. In particular, the composition is characterized
wherein the compound contains a KH-1 or N3 epitope.

 Additionally, the composition is provided wherein
the immunological adjuvant is bacteria or liposomes. The
15 adjuvant may be *Salmonella minnesota* cells, bacille
Calmette-Guerin or QS21.

 Favorably, the composition is provided wherein the
compound has the structure:

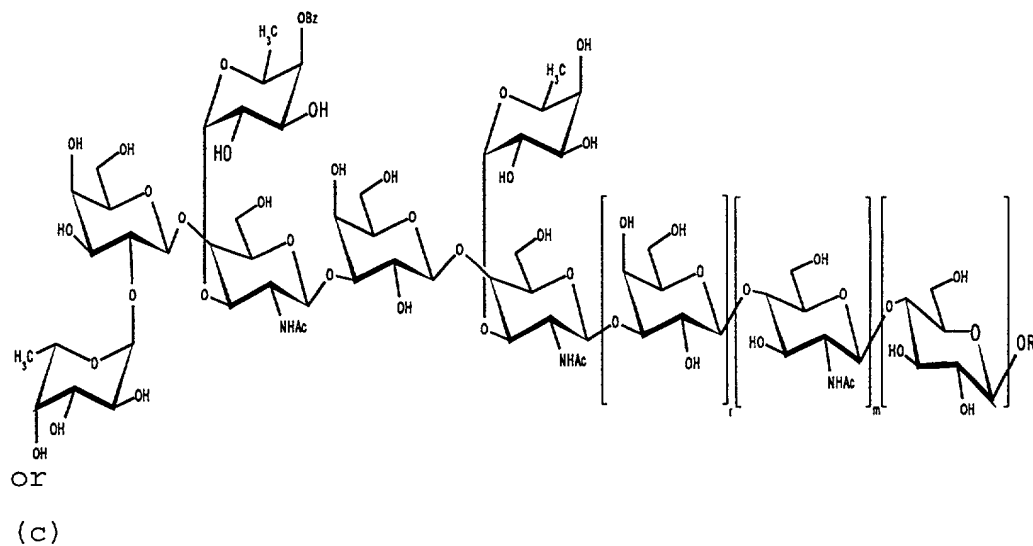
(a)

20

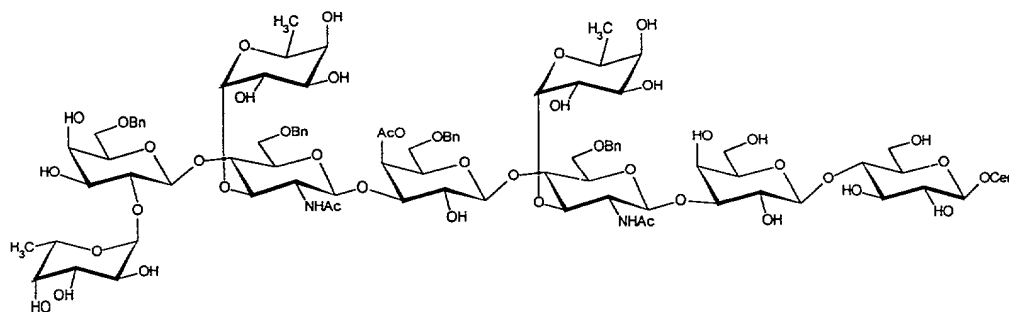


(b)

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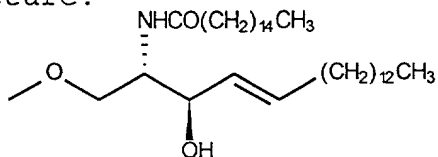


10



wherein R is H, substituted or unsubstituted alkyl, aryl or allyl, or an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, which amino acyl moiety or residue bears an ω -amino group or an ω -(C=O)- group, which group is linked to O via a polymethylene chain having the structure $-(CH_2)_s-$, where s is an integer between about 1 and about 9, or a moiety having the structure:

25



and wherein r, m and n are independently 0, 1, 2 or 3.

Utilities

10 The compounds taught above which are related to
KH-1 and N3 cell-surface antigens are capable of
preventing recurrence of various types of epithelial
cancer in a subject, including lung, gastrointestinal,
prostate and colon cancers, and inducing antibodies
15 useful as a vaccine in the treatment of such types of
cancer, both *in vivo* and *in vitro*. Thus, these antigens
and analogues thereof are useful to treat, prevent or
ameliorate such cancers in subjects suffering therefrom.

20 The magnitude of the therapeutic dose of the
compounds of the invention will vary with the nature and
severity of the condition to be treated and with the
particular compound and its route of administration. In
general, the daily dose range for anticancer activity or
antibody induction lies in the range of 0.001 to 25 mg/kg
25 of body weight in a mammal, preferably 0.001 to 10 mg/kg,
and most preferably 0.001 to 1.0 mg/kg, in single or
multiple doses. In unusual cases, it may be necessary to
administer doses above 25 mg/kg.

30 Any suitable route of administration may be
employed for providing a mammal, especially a human, with
an effective dosage of a compound disclosed herein. For
example, oral, rectal, topical, parenteral, ocular,
pulmonary, nasal, etc., routes may be employed. Dosage

5 forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, etc.

The pharmaceutical compositions of the present invention comprise a compound containing any of the KH-1 and N3 antigens of the subject invention, as an active
10 ingredient, and may also contain a pharmaceutically acceptable carrier and, optionally, other therapeutically active ingredients.

The compositions include compositions suitable for oral, rectal, topical (including transdermal devices, aerosols, creams, ointments, lotions and dusting
15 powders), parenteral (including subcutaneous, intramuscular and intravenous), ocular (ophthalmic), pulmonary (nasal or buccal inhalation) or nasal administration. Although the most suitable route in any given case will depend largely on the nature and severity
20 of the condition being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

In preparing oral dosage forms, any of the unusual pharmaceutical media may be used, such as water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like in the case of oral liquid
25 preparations (e.g., suspensions, elixers and solutions); or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, etc., in the case of oral
30 solid preparations are preferred over liquid oral

5 preparations such as powders, capsules and tablets. If
desired, capsules may be coated by standard aqueous or
non-aqueous techniques. In addition to the dosage forms
described above, the compounds of the invention may be
administered by controlled release means and devices.

10 Pharmaceutical compositions of the present
invention suitable for oral administration may be
prepared as discrete units such as capsules, cachets or
tablets each containing a predetermined amount of the
active ingredient in powder or granular form or as a
15 solution or suspension in an aqueous or nonaqueous liquid
or in an oil-in-water or water-in-oil emulsion. Such
compositions may be prepared by any of the methods known
in the art of pharmacy. In general compositions are
prepared by uniformly and intimately admixing the active
20 ingredient with liquid carriers, finely divided solid
carriers, or both and then, if necessary, shaping the
product into the desired form. For example, a tablet may
be prepared by compression or molding, optionally with
one or more accessory ingredients. Compressed tablets
25 may be prepared by compressing in a suitable machine the
active ingredient in a free-flowing form such as powder
or granule optionally mixed with a binder, lubricant,
inert diluent or surface active or dispersing agent.
Molded tablets may be made by molding in a suitable
30 machine, a mixture of the powdered compound moistened
with an inert liquid diluent.

 The present invention will be better understood
from the Experimental Details which follow. However, one

5 skilled in the art will readily appreciate that the
specific methods and results discussed are merely
illustrative of the invention as described in the claims
which follow thereafter.

10

EXAMPLE 1

15 **6-O-Benzyl-3,4-O-carbonate-galactal (3):** To a solution
of 3,4-carbonate-galactal (5.36 g, 34.37 mmol) in dry DMF
(50 mL) at 0 °C was added benzyl bromide (12.26 mL, 103.0
mmol), followed by NaH (60% oil dispersion, 1.5 gm. 1.1
eq). The reaction was stirred for 1 hr, diluted with
20 CHCl₃ (50 mL) and then brine solution (20 mL) was added
and stirred for 5 min. The organic layer was separated,
dried (MgSO₄), concentrated, and subjected to
chromatographic purification (1:1, Hex:EA) to obtain
compound 3 (85%) as a syrup: $[\alpha]^{23}_D = -92.0$ (c 1.0,
25 CHCl₃); FTIR (thin film) 3030, 2875, 1797, 1647, 1496,
1453, 1371, 1244, 1164, 1110, 1010, 837, 699 cm⁻¹; ¹H
NMR (400 MHz, CDCl₃) δ 3.7-3.9 (m, 2H, H-6), 4.08 (bt, 1H,
J = 7.36 Hz, H-5), 4.58 (s, 2H, -CH₂Ar), 4.90 (d, 1H, J
= 7.76 Hz, H-4), 4.93 (bm, 1H, H-3), 5.14 (dd, 1H, J =
3.16 Hz, J = 7.72 Hz, H-2), 6.66 (d, 1H, J = 6.24 Hz, H-
30 1), 7.28-7.45 (m, 5H, Ar-H); ¹³C NMR (400 MHz, CDCl₃) δ
67.97, 68.74, 72.41, 73.14, 73.66, 97.97, 127.77, 127.93,
128.44, 137.18, 149.06, 153.98.

6-O-Benzylglucal (3'): To a solution of a glucal (10 g, 68.42 mmol) in a dry DMF (200 mL) was added at -40 °C LHMDs (1.0 M soln in THF, 75.26 mL, 1.1 eq) dropwise, followed by BnBr (8.18 mL, 68.42 mmol). The solution was stirred mechanically for 6 hrs allowing the temperature to rise to 0 °C. At room temperature, a sat'd solution of ammonium chloride (50 mL) was added, followed by EtOAc (200 mL). The organic layer was separated; the aqueous layer was extracted with EtOAc (3 x 50 mL). Combined organic layers were washed with brine (50 mL), water (50 mL), dried with (MgSO₄), filtered, concentrated and purified by column chromatography (1:1 Hex: EtOAc) to obtain compound 3' as a syrup: $[\alpha]_D^{23} = +11.0$ (c 1.0, CHCl₃); FTIR (thin film): 3342, 2871, 1642, 1656, 1231, 1101, 1027, 851, 738 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 3.6-3.85 (m, 5H), 4.06 (d, 1H, J = 4.0 Hz, -OH), 4.11 (bt, 1H, H-3), 4.46 (d, 1H, J = 12.0 Hz, -CH₂Ar), 4.52 (d, 1H, J = 12.0 Hz, -CH₂Ar), 4.57 (dd, 1H, J = 1.84 Hz, J = 5.96 Hz, H-2), 6.21 (d, 1H, J = 5.96 Hz, H-1), 7.15-7.35 (m, 5H, Ar-H); ¹³C NMR (400 MHz, CDCl₃) δ 69.06, 69.63, 70.28, 73.427, 76.95, 102.72, 127.29, 127.55, 128.21, 128.24, 137.60, 137.75, 143.86.

5 **6-O-Benzyl-3-O-triethylsilylglucal (4):** To a solution of
compound 3 (5 g, 21.16 mmol) in dry CH₂Cl₂ (50 mL) was
added imidazole (1.72 g, 25.39 mmol), DMAP (10 mg). At
0 °C TESCl (3.90 mL, 23.27 mmol) was added dropwise.
The reaction mixture was stirred for 9 hrs, washed with
10 water (2 x 10 mL) and brine (10 mL). The organic layer
was separated and dried (MgSO₄), concentrated and
purified by column chromatography (20% EA in hexane) to
obtain 4 (5.47 mg ,73 %) as a syrup: $[\alpha]^{22}_D + 44.0$ (c
1.0, CHCl₃); FTIR (thin film) 3468, 3030, 2953, 2875,
15 1644, 1453, 1237, 1086, 871, 737 cm⁻¹; ¹H NMR (400 MHz,
CDCl₃) δ 0.55 (q, 6H, J = 7.90 Hz, -SiCH₂CH₃), 0.88 (t,
9H, J = 7.90 Hz, -SiCH₂CH₃), 2.47 (d, 1H, J = 4.12 Hz,
-OH), 3.6-3.75 (m, 3H, 2H-6, H-4), 4.13 (bd, 1H, J = 6.4
Hz, H-3), 4.47 & 4.52 (2d, 2H, J = 12.00 Hz, -CH₂Ar),
20 4.55 (dd, 1H, J = 2.24 Hz, J = 6.16 Hz, H-2), 6.21 (d,
1H, J = 5.96 Hz, H-1), 7.10-7.40 (m, 5H, Ar-H); ¹³C NMR
(400 MHz, CDCl₃) δ 4.84, 6.66, 69.05, 69.64, 70.56,
73.47, 76.97, 103.44, 127.59, 127.64, 128.27, 137.78,
143.33.

25

EXAMPLE 4

30 **3,6,6'-Tri-O-benzyl-4',5'-carbonate-lactal (7):** To a
solution of compound 3 (3.00 gm, 11.43 mmol) in a dry
CH₂Cl₂ (20 mL) at 0 °C was added 3,3-dimethyldioxirine
(300 mL, 0.08 M solution in acetone). The reaction was

5 stirred at 0 °C for 1h. The organic solvent was
evaporated in a stream of N₂ gas. The residue was dried
in vacuum for 10 minutes. The resulting anhydro sugar
was dissolved in a solution of the compound 3,6-
dibenzylglucal (5.29 gm, 17.15 mmol) in a dry THF (30
10 mL). At 0 °C a 1.0 M solution of ZnCl₂ in ether (5.71
mL, 0.5 eq) was added. The reaction was stirred at room
temperature for 24 h, diluted with EtOAc (50 mL), washed
with a sat'd solution of NaHCO₃ (2 x 10 mL). The organic
layer was separated, dried (MgSO₄) and purified by
15 chromatography using EA:Hexane (1:1) to obtain compound
7, 3.3 g (48 %) (60 % wrt recovered SM) as a syrup:
[α]²²_D - 38.0 (c 1.0, CHCl₃); FTIR (thin film) 3437,
3029, 2871, 1804, 1648, 1453, 1367, 1166, 1097, 1027,
739, 697 cm⁻¹; ¹H NMR (400 Mhz, CDCl₃) δ 3.55-3.62 (m,
20 2H), 3.62-3.70 (m, 2H), 3.70-3.78 (m, 2H), 3.95-4.11 (m,
2H), 3.95-4.11 (m, 2H), 4.17 (dd, 1H, J = 5.36 Hz, J =
7.04), 4.27 (ddd, 1H, J = 1.12 Hz, J = 1.73 Hz, J = 5.29
Hz), 4.44 (s, 2H, -CH₂Ar), 4.77 (dd, 1H, J = 2.48 Hz, J
= 6.12 Hz, H-2), 6.28 (d, 1H, J = 6.04 Hz, H-1), 7.10-
25 7.40 (m, 15H, Ar-H); ¹³C NMR (400 MHz, CDCl₃) δ 68.00,
68.09, 70.55, 70.63, 72.20, 73.58, 73.81, 74.58, 74.82,
75.26, 76.18, 78.47, 100.17, 101.32, 127.43 (2C), 127.56,
127.72 (2C), 127.83, 127.90, 128.00 (2C), 128.31(2C),
128.37 (2C), 128.44 (2C), 137.28, 137.43, 138.29, 144.59,
30 153.97.

EXAMPLE 5

3,6,6'-Tri-O-benzyl-lactal (10): To a solution of compound 7 (3.00 g, 4.96 mmol) in MeOH (100 mL) was added dropwise a solution of sodium methoxide (1 mL, 25 % by wt in MeOH). The reaction was stirred for 1h, and the solvent was evaporated. The syrup obtained was rapidly purified by column chromatography (2.5 % MeOH in EtOAc) to obtain 2.68 g (91 %) of 10 as syrup: $[\alpha]_D^{22}$ -14.0 (c 1.0, CHCl₃); FTIR (thin film) 3415, 3029, 2867, 1647, 1453, 1246, 1068, 735 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 3.48-3.56 (m, 2H), 3.62 (dd, 1H, J = 4.80 Hz, J = 8.0 Hz), 3.66-3.78 (m, 3H), 3.91 (d, 1H, J = 4.4 Hz), 3.97 (dd, 1H, J = 4.0 Hz, J = 8.8 Hz), 4.18-4.28 (m, 4H), 4.47 (s, 2H, -CH₂Ar), 4.52 (d, 1H, J = 8.0 Hz), 5.59 (s, 1H, -CH₂Ar), 4.57-4.65 (m, 2H, -CH₂Ar), 4.85 (dd, 1H, J = 2.4 Hz, J = 4.8 Hz, H-2), 6.41 (d, 1H, J = 4.8 Hz, H-1), 7.20 - 7.45 (m, 15H, Ar-H); ¹³C NMR (400 MHz, CDCl₃) δ 67.92, 68.86, 69.19, 69.82, 71.53, 73.35(2C), 73.39, 73.42, 73.87, 76.26, 100.01, 103.30, 127.35, 127.42, 127.59, 127.74, 128.19, 128.29, 137.72, 137.81, 138.52, 144.57.

EXAMPLE 6

30

Monosilylated lactal (6): To a solution of compound 3 (3.00 gm, 11.43 mmol) in a dry CH₂Cl₂ (20 mL) at 0 °C was added 3,3-dimethyldioxirine (300 mL, 0.08 M solution in

5 acetone). The reaction was stirred at 0 °C for 1h, and
the organic solvents were evaporated in a N₂ gas stream.
The residue was dried in vacuum for 10 minutes. The
resulting anhydro sugar was dissolved in a solution of
10 compound 4 (6 gm, 9.2 mmol) in a dry THF (30 mL), at 0 °C
was added a 1.0 M solution of ZnCl₂ in ether (6 g, 0.5
eq). Reaction was stirred at room temperature for 24 h.
Diluted with EtOAc (50 mL), washed with sat. solution of
NaHCO₃ (2 x 10 mL), organic layer was separated, dried
15 (MgSO₄) submitted for chromatography EA: Hexane (2:3) to
obtain compound 6 (4.8 g 66 %) (81 % wrt recovered 7) as
a syrup: $[\alpha]_D^{22}$ -25.0 (c 1.0, CHCl₃); IR (thin film)
3439, 3030, 2910, 1804, 1725, 1647, 1453, 1371, 1243,
1074, 847, 741 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 0.58 (q,
6H, J = 8.0 Hz, -SiCH₂CH₃), 0.92 (t, 9H, J = 8.0 Hz, -
20 SiCH₂CH₃), 3.51 (d, 1H, J = 2.8 Hz, -OH), 3.62 (ddd, 1H,
J = 2.8 Hz, J = 7.2 Hz, J = 7.2 Hz, H-2'), 3.65-3.75 (m,
3H), 3.85 (m, 1H), 3.93 (dd, 1H, J = 4.92 Hz, J = 11.24
Hz), 3.99 (bt, 1H, J = 5.32 Hz, J = 6.48 Hz), 4.09 (bm,
1H), 4.27 (bt, 1H, J = 4.16 Hz), 4.48-4.68 (m, 6H, -
25 CH₂Ar), 4.70 (dd, 1H, J = 3.36 Hz, J = 6.16 Hz, H-2),
4.74 (dd, 1H, J = 1.8 Hz, J = 7.16 Hz, H-4), 6.32 (d, 1H,
J = 6.04, H-1), 7.2-7.4 (m, 10H, Ar-H); ¹³C (500 MHz,
CDCl₃) δ 4.75, 6.67, 65.65, 67.79, 67.93, 70.42, 71.49,
73.43, 73.58, 74.46, 75.27, 75.42, 78.05, 99.94, 102.61,
30 127.79, 127.85, 128.14, 128.33, 137.36, 137.53, 143.00,
153.96.

Acetylated silyl lactal: To a solution of compound 6 (3.5 g, 5.50 mmol) in CH_2Cl_2 (30 mL) was added pyridine (3 mL), Ac_2O (3 mL) and DMAP (cat). The reaction was stirred overnight, and then diluted with EtOAc (50 mL), washed with a sat'd solution of CuSO_4 (3 x 10 mL), water (1 x 10 mL), NaHCO_3 (2 x 10 mL), and brine (1 x 10 mL). The organic layer was separated, dried, and concentrated. The residue was purified by chromatography (1:1, Hex:EA) to obtain 6' in quantitative yield; $[\alpha]^{23}_{\text{D}} - 42.0$ (*c* 1.0, CHCl_3); IR (film) 2954, 2875, 1809, 1755, 1646, 1454, 1222, 1060, 743 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.58 (q, 6H, $J = 7.92$ Hz, $-\text{SiCH}_2\text{CH}_3$), 0.92 (t, 9H, $J = 7.92$ Hz, $-\text{SiCH}_2\text{CH}_3$), 2.06 (s, 3H, $-\text{COCH}_3$), 3.63 (dd, 1H, $J = 2.92$ Hz, $J = 10.92$ Hz, H-5), 3.70 (bd, 2H, $J = 10.52$ Hz, 2H-6), 3.85 (dd, 1H, $J = 6.08$ Hz, $J = 10.92$ Hz, H-5'), 3.9-4.0 (m, 2H, 2H-6'), 4.10 - 4.2 (m, 2H), 4.5-4.6 (m, 4H, 2- CH_2Ar), 4.64 (dd, 1H, $J = 3.96$ Hz, 8.0 Hz, H-3'), 4.71 (dd, 1H, $J = 4.24$ Hz, $J = 5.84$ Hz, H-2), 4.84 (dd, 1H, $J = 1.04$ Hz, $J = 8.08$ Hz, H-4'), 4.90 (d, 1H, $J = 4.60$ Hz, H-1'), 4.99 (t, 1H, $J = 4.20$ Hz, H-4'), 6.30 (d, 1H, $J = 6.16$ Hz, H-1), 7.15 - 7.40 (m, 10H, Ar-H); ^{13}C NMR (CDCl_3) δ 4.78, 6.73, 20.58, 64.78, 67.94, 67.99, 69.38, 69.50, 73.19, 73.35, 73.79, 73.92, 74.56, 74.86, 96.82, 102.27, 127.60, 127.75, 127.78, 127.93, 128.29, 128.44, 137.35, 138.01, 142.99, 153.27, 168.54.

EXAMPLE 8

Iodosulfonamide (6''): To a solution of compound 6' (2.5 gm, 3.72 mmol) (suspended with 4 A MS (3.00 g)) and benzenesulfonamide (2.92 g, 18.57 mmol) at 0 °C, was added (via cannula) a solution of $I^+(coll)_2ClO_4^-$ (freshly prepared from $Ag(coll)ClO_4$ (8.36 g, 18.59 mmol) and I_2 (4.53 g, 18.53 mmol)) in CH_2Cl_2 (40 mL). The reaction mixture was allowed to warm to r.t. and stirred for 1hr. The mixture was filtered through a pad of silica gel. The filtrate was washed with a sat'd solution of $Na_2S_2O_3$ (3 x 25 mL), followed by a sat'd solution of $CuSO_4$ (5 x 25 mL), and H_2O (2 x 10 mL). The organic layer was separated and dried ($MgSO_4$), concentrated and purified by column chromatography (5 % EA in CH_2Cl_2 , in a gradient elution) to obtain 6'', 2.9 g (81 %) as a syrup; $[\alpha]_D^{23}$ -30.0 (c 1.0, $CHCl_3$); IR (film) 3267, 2954, 1806, 1755, 1495, 1458, 1370, 1342, 1090, 813, 750 cm^{-1} ; 1H NMR ($CDCl_3$) δ 0.66 (m, 6H, $J = 7.8$ Hz, $-SiCH_2CH_3$) 0.95 (t, 9H, $J = 7.8$ Hz, $-SiCH_2CH_3$), 2.04 (s, 3H, $-COCH_3$), 3.44 (dd, 1H, $J = 5.56$ Hz, $J = 10.16$ Hz, H-5), 3.55-3.72 (m, 4H), 3.86 (bs, 1H), 4.11 (t, 1H, $J = 6.96$ Hz), 4.23 (bs, 1H), 4.35 (dd, 1H, $J = 2.28$ Hz, $J = 9.98$ Hz), 4.44 & 4.50 (2d, 2H, 11.88 Hz, $-CH_2Ar$), 4.57 (s, 2H, $-CH_2Ar$), 4.70 (bd, 1H, $J = 8.32$ Hz), 4.89 (bs, 1H), 4.95-5.0 (m, 2H), 5.25 (t, 1H, $J = 9.64$ Hz), 5.60 (d, 1H, $J = 9.92$ Hz), 7.2-7.5 (m, 13H, Ar-H), 7.88 (d, 2H, $J = 7.72$ Hz, Ar-H);

5 ^{13}C NMR (CDCl_3) δ 4.93, 6.95, 20.64, 67.49, 67.86, 68.40, 68.46, 71.91, 72.57, 73.33, 73.94, 75.20, 79.30, 126.39, 127.35, 127.67, 127.85, 127.98, 128.09, 128.36, 128.54, 128.58, 129.10, 132.35, 132.68, 137.14, 137.91, 141.36, 153.60, 168.69.

10

EXAMPLE 9

15 **Thiodonor (9):** To a solution of iodosulfonamide 6'' (2.8 g, 2.93 mmol) in dry DMF (40 mL) at -40°C was added EtSH (1.08 mL, 14.65 mmol), followed by dropwise addition of a solution of LHMDS (1.0 M solution in THF, 8.80 mL). The reaction mixture was stirred for 1 hr while allowing it to warm up to r.t., and then neutralized with a

20 saturated solution of NH_4Cl (10 mL), and extracted with EtOAc (5 x 20 mL). The organic layer was washed with brine (15 mL), separated, dried (MgSO_4), and concentrated. The resulting residue was acetylated in CH_2Cl_2 (50 mL) with pyridine (1.0 mL), 2Ac O (1.0 mL)

25 overnight. The organic layer was washed with a sat'd solution of CuSO_4 (3 x 15 mL), water (1 x 10 mL), and a sat'd solution of NaHCO_3 (2 x 15 mL). The organic layer was separated, dried (MgSO_4) and concentrated. The residue was purified by chromatography (1:1, Hex: EA) to

30 obtain 9 (2.38 g, 91%) as syrup; $[\alpha]_D^{23} - 4.0$ (c 1.0, CHCl_3); IR (film) 3316, 2955, 2875, 1815, 1745, 1448, 1371, 1330, 1227, 1092, 897, 740 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.51 (q, 6H, $J = 8.0\text{ Hz}$, $-\text{SiCH}_2\text{CH}_3$), 0.88 (t, 9H, $J =$

5 7.92 Hz, -SiCH₂CH₃), 1.09 (t, 3H, 7.20 Hz, -SCH₂CH₃),
 2.09 (s, 3H, -COCH₃), 2.44 (m, 2H, -SCH₂CH₃), 3.48 (bm,
 1H, H-2), 3.83-3.70 (m, 7H), 3.89 (bt, 1H), 3.95 (bs,
 1H), 4.43 (d, 1H, J = 5.44 Hz, H-1), 4.48 (bd, 2H, -
 CH₂Ar), 4.53 (d, 1H, J = 6.32 Hz, H-1'), 4.57 (s, 2H, -
 10 CH₂Ar), 4.75 (bt, 1H, J = 5.72 Hz, H-2'), 4.84 (bd, 1H,
 9.88 Hz, -NHSO₂Ph), 7.20-7.40 & 7.40-7.60 (m, 13H, Ar-
 H), 7.97 (d, 2H, J = 7.16 Hz, Ar-H); ¹³C NMR (CDCl₃) δ
 4.28, 6.65, 14.56, 20.64, 20.89, 56.96, 67.64, 70.49,
 70.52, 70.57, 71.14, 73.26, 73.72, 73.96, 74.99, 75.02,
 15 76.88, 82.48, 97.83, 126.21, 127.30, 127.61, 127.78,
 127.87, 128.29, 128.38, 128.62, 128.94, 132.17, 137.32,
 137.94, 141.38, 153.27, 170.99.

20

EXAMPLE 10

Disilylated lactal (6'''): To a solution of lactal 6 (3
 gm, 4.77 mmol) in dry CH₂Cl₂ (50 mL) at 0 °C, was added
 25 Et₃N (3.34 mL), followed by the dropwise addition of
 TESOTf (1.61 mL, 7.15 mL). The reaction mixture was
 stirred for 3 h, and washed with a sat'd solution of
 NaHCO₃ (2 x 15 mL). The organic layer was separated,
 dried (MgSO₄), and concentrated. The residue was
 30 purified by chromatography (4:1, Hex:EA) to obtain 6'''
 (3.27 g, 92%) as a syrup; [α]_D²⁵ -38.0 (c 1.0, CHCl₃); IR
 (thin film) δ 3087, 2953, 2875, 1819, 1647, 1647, 1454,
 1365, 1240, 1101, 854, 739 cm⁻¹; ¹H NMR (CDCl₃, 400MHz)

5 0.57 & 0.617 (2q, 12H, J = 8.0 Hz, -SiCH₂CH₃), 0.92 &
 0.94 (2t, 18H, J = 8.0 Hz, -SiCH₂CH₃), 3.5-3.75 (m, 4H),
 3.8-4.0 (m, 3H), 4.05-4.20 (m, 2H), 4.49 (dd, 1H, J =
 4.36 Hz, J = 7.24 Hz), 4.50-4.62 (m, 4H, -CH₂Ar), 4.64
 (d, 1H, J = 5.2 Hz, H-1'), 4.70 (dd, 1H, J = 4.0 Hz, J =
 10 5.60 Hz, H-4'), 4.76 (bd, 1H, J = 7.5 Hz, H-2), 6.32 (d,
 1H, J = 6.0 Hz, H-1); ¹³C NMR (CDCl₃, 400 MHz) δ 4.56,
 4.79, 6.58, 6.76, 65.24, 67.99, 68.02, 69.48, 71.06,
 73.37, 73.76, 74.24, 74.37, 75.10, 78.21, 99.21, 99.34,
 102.56, 127.63, 127.77, 127.79, 127.88, 128.32, 128.43,
 15 137.53, 138.09, 143.08, 153.87.

20

EXAMPLE 11

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30

Disilylated Iodosulfonamide (6'''): To a solution of
 lactal 6''' (2.5 g, 3.36 mmol) (suspended with 4 Å MS (3
 g)) and benzenesulfonamide (2.64 g, 3.36 mmol) at 0°C,
 was added a freshly prepared solution of I(sym-coll)₂ClO₄
 (5eq) in CH₂Cl₂. The reaction mixture was stirred at r.t.
 for 1hr, filtered through a pad of silica gel, washed
 with a sat'd solution of Na₂S₂O₃ (3 x 25 mL), CuSO₄ (5 x
 25 mL), and water (2 x 10 mL). The organic layer was
 separated, dried (MgSO₄), and concentrated. The
 resulting residue was purified by chromatography (5% EA
 in CH₂Cl₂) to obtain 6''' (3.20 g, 92%) as a syrup;
 [α]²³_D -19.0 (c 1.0, CHCl₃); IR (thin film) 3258, 2953,
 2875, 1806, 1788, 1453, 1331, 1105, 849, 745 cm⁻¹; ¹H

5 NMR (CDCl₃, 400 MHz) δ -0.57 & 0.64 (2q, 12H, J = 8.0
Hz, -SiCH₂CH₃), 0.90 & 0.95 (2t, 18H, J = 8.0 Hz, -
SiCH₂CH₃), 3.39 (bm, 1H, H-2), 3.60 - 3.70 (m, 4H), 3.78-
3.83 (bm, 2H), 4.05-4.17 (m, 3H), 4.34 (dd, 1H, J = 2.40
10 Hz, J = 8.68 Hz), 4.45 & 4.52 (2d, 2H, J = 12.0 Hz, -
CH₂Ar), 4.55 (s, 2H, -CH₂Ar), 4.68 (d, 1H, J = 2.96 Hz),
4.89 (d, 1H, J = 8.56 Hz), 5.29 (t, 1H, J = 8.36 Hz),
5.47 (d, 1H, J = 9.64 Hz, -NH₂SO₂Ph), 7.2-7.5 (m, 13H, Ar-
H), 7.89 (d, 2H, J = 7.6 Hz, Ar-H); ¹³H NMR (CDCl₃, 400
15 MHz) δ 4.54, 4.94, 6.59, 6.95, 67.94, 68.12, 68.39,
68.63, 73.12, 73.31, 73.36, 73.90, 75.26, 75.33, 76.86,
79.66, 100.04, 127.40, 127.67, 127.76, 127.93, 128.01,
128.36, 128.51, 128.60, 132.39, 137.34, 138.02, 141.31,
154.01.

20

EXAMPLE 12

Disilylated thiodoner (8): To a solution of
iodosulfonamide 6''' (2.7 g, 2.63 mmol) in dry DMF (40 mL)
25 at -40 °C, was added EtSH (0.584 mL, 7.89 mmol), followed
by the dropwise addition of a solution of LHMDs (1.0 M
solution in THF, 7.89 mL). The reaction mixture was
stirred for 1 hr while allowing it to warm up to r.t.,
and then neutralized with a saturated solution of NH₄Cl
30 (10 mL). EtOAc was added (50 mL). The organic layer was
washed with brine (5 mL), separated, dried (MgSO₄), and
concentrated. The residue was purified by chromatography

5 (7:3, Hex:EA) to obtain 8 (2.3 g, 91%) as syrup; $[\alpha]_D^{23}$ -64.0 (c 1.0, CHCl_3); IR (thin film) 3314, 2954, 2875, 1807, 1453, 1330, 1181, 1104, 739 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 0.50 (q, 6H, $J = 7.88$ Hz, $-\text{SiCH}_2\text{CH}_3$), 0.624 (q, 6H, $J = 7.6$ Hz, $-\text{SiCH}_2\text{CH}_3$), 0.87 (t, 9H, $J = 7.88$ Hz, $-\text{SiCH}_2\text{CH}_3$), 0.94 (t, 9H, $J = 7.96$ Hz, $-\text{SiCH}_2\text{CH}_3$), 1.11 (t, 3H, $J = 7.44$ Hz, $-\text{SCH}_2\text{CH}_3$), 2.48 (m, 2H, $-\text{SCH}_2\text{CH}_3$), 3.35 (m, 1H, H-2), 3.85-3.68 (m, 6H), 3.86 (bm, 1H), 3.97 (bt, 1H), 4.06 (bt, 1H, $J = 6.56$ Hz), 4.49 (s, 2H, $-\text{CH}_2\text{Ar}$), 4.57 (s, 2H, $-\text{CH}_2\text{Ar}$), 4.55 (m, 1H), 4.61 (d, 1H, $J = 6.28$ Hz, H-1), 4.67 (d, 1H, $J = 4.0$ Hz, H'-4), 4.87 (d, 1H, $J = 8.84$ Hz, H-1), 5.50 (d, 1H, $J = 8.84$ Hz, $-\text{NH}\text{SO}_2\text{Ph}$), 7.2-7.4 (m, 10H, Ar-H), 7.45-7.55 (m, 3H, Ar-H), 7.94 (d, 2H, $J = 7.2$ Hz, Ar-H); ^{13}C NMR (CDCl_3) δ 4.28, 4.45, 6.56, 6.71, 14.58, 25.64, 57.42, 67.82, 69.05, 69.68, 70.37, 71.80, 73.13, 73.66, 73.75, 76.45, 76.64, 77.05, 82.68, 100.94, 127.52, 127.57, 127.79, 127.83, 128.22, 128.35, 128.84, 132.25, 137.49, 138.01, 140.70.

25

EXAMPLE 13

Tetrasaccharide diol (9'): To a solution of disaccharide 10 (100 mg, 0.173 mmol) and thiodonor 9 (308 mg, 0.34 mmol) in dry CH_2Cl_2 (8 mL), suspended with 4 Å MS (1.0 g) was added di-*t*-butylpyridine (0.311 mL, 1.36 mmol), cooled to -10°C . Then, MeOTf (0.156 mL, 1.36 mmol) was added. The reaction mixture was stirred for 2 h, then at

5 0 °C for 24 h. After neutralizing with Et₃N (0.1 ml),
the mixture was diluted with EtOAc (25 mL), and filtered
through a pad of silica gel. The filtrate was washed
with a sat'd solution of NaHCO₃ (2 x10 mL). The organic
layer was separated, dried (MgSO₄), and concentrated.
10 The residue was purified by chromatography to obtain
tetrasaccharide 9' in 55% as syrup; $[\alpha]_D^{23}$ -28.0 (c 1.0,
CHCl₃); I.R. (film) 3491, 3029, 3874, 1815, 1753, 1647,
1453, 1370, 1221, 1160, 1064, 738 cm⁻¹; ¹H NMR (CDCl₃)
δ 0.38 (q, 6H, J = 7.96 Hz, -SiCH₂CH₃), 0.76 (t, 9H, J =
15 7.96 Hz, -SiCH₂CH₃), 1.97 (s, 3H, -COCH₃), 3.2-3.32 (m,
2H), 3.35-3.55 (m, 5H), 3.55-3.7 (m, 7H), 3.7-3.8 (m,
4H), 3.95 (dd, 1H, J = 4.64 Hz, J = 11.28 Hz), 4.0 - 4.12
(m, 2H), 4.18 (bs, 1H), 4.3-4.65 (m, 15H), 4.7-4.8 (m,
2H), 4.89 (t, 1H, J = 5.24 Hz), 5.31 (d, 1H, J = 8.4 Hz),
20 6.32 (d, 1H, J = 6.04 Hz, H-1), 7.1- 7.5 (m, 28H, Ar-H),
7.85 (d, 2H, J = 7.4 Hz, Ar-H); ¹³C NMR (CDCl₃) δ 4.47,
6.74, 20.62, 58.45, 67.89, 68.16, 68.91, 69.63, 70.01,
70.23, 70.70, 70.74, 72.95, 73.26, 73.32, 73.40, 73.43,
73.79, 74.38, 74.75, 74.81, 75.34, 76.60, 77.19, 82.21,
25 97.41, 100.41, 102.53, 102.84, 127.30, 127.44, 127.50,
127.56, 127.59, 127.62, 127.76, 127.82, 127.84, 127.96,
128.18, 128.24, 128.33, 128.38, 128.46, 128.83, 132.49,
137.32, 137.89, 138.70, 140.76, 144.51, 153.43, 168.94.

30

EXAMPLE 14

5 **Tetrasaccharide pentaol (11):** To a solution of
tetrasaccharide 9' (370 mg, 0.26 mmol) in MeOH (5 mL) was
added K₂CO₃ (370 mg). The reaction mixture was stirred
for 15 min, diluted with CH₂Cl₂ (100 mL), and filtered
10 through a pad of silica gel, followed by washing with
EtOAc (100 mL). The filtrates were combined, and
concentrated to obtain 11 (295 mg, 85%) as a syrup;
[α]²³_D -18.0 (c 1.0, CHCl₃); IR (film) 3469, 3030, 2873,
1648, 1496, 1452, 1328, 1092, 909, 737 cm⁻¹; ¹H NMR
(CDCl₃) δ 0.31 (q, 6H, J = 6.38 Hz, -SiCH₂CH₃), 0.70 (t,
15 9H, J = 6.38 Hz, -SiCH₂CH₃), 2.49 (bs, 1H, -OH), 2.82
(bs, 1H, -OH), 3.16 (m, 1H, -CHNHSO₂Ph), 3.3-3.6 (m,
12H), 3.6-3.78 (m, 6H), 3.79 (bs, 2H), 3.8-3.85 (m, 3H),
3.92 (bd, 1H, J = 4.23 Hz), 4.0 (bt, 1H), 4.05-4.10 (m,
2H), 4.10-4.25 (m, 3H), 4.30-4.40 (m, 6H), 4.4-4.55 (m,
20 7H), 4.75 (dd, 1H, J = 2.73 Hz, J = 4.94 Hz), 4.9 (d, 1H,
J = 4.20 Hz), 6.17 (d, 1H, 6.63 Hz, -HNSO₂Ph), 6.31 (d,
1H, J = 4.9 Hz, H-1), 7.0-7.4 (m, 23H, Ar-H), 7.80 (d, 2H,
J = 6.00 Hz, Ar-H); ¹³C NMR (CDCl₃) δ 4.30, 6.72, 57.88,
68.00, 68.78, 68.84, 69.20, 70.46, 70.86, 71.39, 71.99,
25 73.04, 73.14, 73.31, 73.40, 73.54, 73.79, 75.72, 76.01,
76.16, 81.44, 100.15, 101.85, 102.32, 102.60, 127.30,
127.45, 127.58, 127.62, 127.65, 127.73, 128.17, 128.21,
128.30, 128.33, 128.90, 132.52, 137.71, 137.87, 137.91,
138.10, 138.62, 140.18, 144.27.

30

EXAMPLE 15

Hexasaccharide tetrol (15'): To a solution of disaccharide **8** (197 mg, 0.20 mmol) and tetrasaccharide **15** (275 mg, 0.20 mmol) in $\text{CH}_2\text{Cl}_2:\text{Et}_2\text{O}$ (1:2, 15 mL) (suspended with 4 Å molecular sieves (1.20 g)) and di-*t*-butylpyridine (0.184 mL, 0.80 mmol) at -10°C was added MeOTf (0.092 mL, 0.80 mmol). The reaction mixture was stirred for 2 h, allowed to warm up to 0°C . After stirring for 24 h, the mixture was diluted with EtOAc (15 mL), filtered through a pad of silica gel, and washed with a sat'd solution of NaHCO_3 (2 x 10 mL). The organic layer was separated, dried (MgSO_4), and concentrated. The residue was purified by chromatography (1:1, Hex:EA) to obtain **15'** (276 mg, 60%) as a syrup; $[\alpha]^{23}_{\text{D}} -23.0$ (c, 1.0, CHCl_3); I.R. (film) 3490, 3030, 2875, 1807, 1649, 1453, 1330, 1093, 909, 743 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.25 (m, 6H, $-\text{SiCH}_2\text{CH}_3$), 0.37 (q, 6H, $J = 7.92$ Hz, $-\text{SiCH}_2\text{CH}_3$), 0.71 (t, 9H, $J = 7.88$ Hz, $-\text{SiCH}_2\text{CH}_3$), 0.76 (t, 9H, $J = 7.92$ Hz, $-\text{SiCH}_2\text{CH}_3$), 0.86 (t, 9H, $J = 7.88$ Hz, $-\text{SiCH}_2\text{CH}_3$), 2.46 (s, 1H, $-\text{OH}$), 2.52 (s, 1H, $-\text{OH}$), 3.15 (m, 1H, $-\text{CHNHSO}_2\text{Ph}$), 3.21 (m, 1H, $-\text{CHNHSO}_2\text{Ph}$), 3.28 (dd, 1H, $J = 3.04$, $J = 9.24$ Hz), 3.37-3.55 (m, 7H), 3.55-3.79 (m, 14H), 3.82 (bs, 2H), 3.89 (bs, 1H), 3.94-4.11 (m, 4H), 4.18 (bs, 1H), 4.28 (m, 1H), 4.33-4.40 (m, 3H), 4.41 (s, 2H, $-\text{CH}_2\text{Ar}$), 4.44-4.47 (m, 3H), 4.49 (s, 2H, $-\text{CH}_2\text{Ar}$), 4.52 (m, 1H), 4.54 (s, 2H, $-\text{CH}_2\text{Ar}$), 4.55-4.63 (m, 2H), 4.66 (dd, 2H, $J = 3.88$ Hz, $J = 6.04$ Hz), 4.74 (dd, 1H, $J = 2.76$ Hz, $J = 6.08$ Hz), 5.28 (d, 1H, $J = 7.52$ Hz, $-\text{NHSO}_2\text{Ph}$), 5.51 (d, 1H, $J = 8.32$ Hz, $-\text{NHSO}_2\text{Ph}$), 6.32 (d,

5 1.90 (s, 3H, -COCH₃), 2.08 (s, 2H, -COCH₃), 2.15 (s, 3H,
 -COCH₃), 3.03 (bd, 1H, J = 7.68 Hz, -CHNHSO₂Ph), 3.2-3.4
 (m, 8H), 3.4-3.85 (m, 30H), 3.85-4.2 (m, 8H). 4.20-4.6
 (m, 29H), 4.75 (q, 1H, J = 3.12 Hz, 6.0 Hz), 4.8 (bd, 1H,
 10 J = 8.2 Hz, 4.88 (d, 1H, J = 3.48 Hz), 5.10 (m, 2H, J =
 8.76 Hz), 5.26 (d, 1H, J = 2.52 Hz), 5.33 (d, 1H, J =
 8.68 Hz, -NHSO₂Ph), 5.42 (d, 1H, J = 2.64 Hz), 5.90 (d,
 1H, J = 10.84 Hz, -NHSO₂Ph), 6.31 (d, 1H, 6.0 Hz, H-1),
 7.1-7.5 (m, 41H, Ar-H), 7.82 & 7.89 (2bm, 4H, Ar-H); ¹³H
 NMR (CDCl₃) δ 4.10, 4.14, 4.49, 6.52, 6.60, 6.64, 20.75,
 15 20.81, 20.09, 21.46, 55.97, 56.73, 67.83, 68.41, 68.63,
 68.80, 69.35, 69.82, 69.88, 70.12, 70.49, 71.09, 71.20,
 71.71, 72.84, 72.95, 73.11, 73.38, 73.53, 73.60, 73.67,
 73.74, 73.79, 74.10, 74.33, 74.40, 75.32, 75.78, 75.89,
 76.18, 76.77, 77.20, 99.75, 100.15, 100.38, 100.53,
 20 101.55, 102.17, 127.26, 127.34, 127.42, 127.47, 127.52,
 127.58, 127.61, 127.62, 127.66, 127.73, 127.73, 127.80,
 127.85, 128.14, 128.21, 128.26, 128.39, 128.41, 128.66,
 128.99, 131.93, 132.60, 137.47, 137.66, 137.77, 137.92,
 138.31, 138.43, 138.77, 139.96, 141.74, 144.48, 154.07,
 25 169.44, 169.60, 169.64, 171.34.

EXAMPLE 17

Hexasaccharide triol (13): To a solution of
 hexasaccharide 12 (175 mg, 0.0725 mmol) in dry THF (5 mL)
 30 was added a solution of TBAF (1.0 M in THF): AcOH (0.725
 mL, 10 eq). The reaction mixture was stirred at 35°C for
 24 h, diluted with EtOAc (10 mL), and washed with a
 saturated solution of NaHCO₃ (2 x 5 mL). The organic

5 layer was separated, dried (MgSO_4), and concentrated.
The residue was purified by chromatography (1:4, Hex:EA)
to obtain **13** (143 mg, 93%) as a white glassy substance;
 ^1H NMR (CDCl_3) δ 1.88, 1.92, 2.01, 2.02 (4s, 3H each, -
COCH₃), 2.85 (bt, 1H, J = 8.24 Hz, -CHNHSO₂Ph), 3.02 (bq,
10 1H, J = 7.0 Hz, -CHNHSO₂Ph), 3.20 (dd, 1H, J = 7.56 Hz, J
= 8.0 Hz), 3.27 (dd, 2H, J = 4.72 Hz, J = 10.00 Hz), 3.3-
3.8 (m, 36H), 3.87 (bs, 2H), 4.03 (bd, 3H), 4.10 (bs,
1H), 4.2-4.65 (m, 33H), 4.66 (d, 1H, 5.1Hz), 4.77 (q, 1H,
 J = 3.2Hz), 5.01 (dd, 1H, J = 8.32 Hz, J = 9.68 Hz), 5.12
15 (dd, 1H, J = 8.2 Hz, J = 9.84 Hz), 5.25 (d, 1H, J = 3.16
Hz), 5.39 (d, 1H, J = 3.08), 6.32 (d, 1H, J = 6.12 Hz, H-
1), 7.10-7.45 (m, 41H, Ar-H), 7.78 (m, 4H, Ar-H);

20

EXAMPLE 18

25

30

Nonasaccharide (15): To a solution of hexasaccharide **13**
(140 mg, 0.067 mmol) and β -flourofucose **14** (241 mg, 0.53
mmol) in dry toluene (10 mL) (suspended with 4 Å
molecular sieves) at 0°C, was added di-*t*-butylpyridine
(0.152 mL, 0.67 mmol) followed by a solution of $\text{Sn}(\text{OTf})_2$
(0.223 mg, 0.53 mmol) in THF (1 mL). The reaction
mixture was stirred for 36 h, and then diluted with EtOAc
(25 mL), and filtered through a pad of silica gel. The
organic layer was washed with a sat'd solution of NaHCO_3
(2 x 10 mL). The organic layer was separated, dried
(MgSO_4), and concentrated. The residue was purified by
chromatography (1:1, Hex:EA) to obtain **15** (135 mg, 60%)

5 as a syrup; ^1H NMR (CDCl_3) δ 0.87 (d, 3H, $J = 6.24$, -
CH₃),), 0.93 (d, 3H, $J = 6.32$ Hz, -CH₃), 1.07 9d, 3H, J
= 6.36 Hz, -CH₃), 1.61 9s, 3H, -COCH₃), 1.80 (s, 3H, -
COCH₃), 1.93 (s, 3H, -COCH₃), 1.98 (s, 3H, -COCH₃), 3.2-
3.4 (m, 6H), 3.4-3.9 (m, 27H), 3.9-4.0 (m, 2H), 4.0-4.2
10 (m, 8H), 4.2-4.65 (m, 34H), 4.65-4.8 (m, 7H), 4.84 (d,
1H, $J = 5.3$ Hz), 4.89 (bt, 1H, $J = 8.4$ Hz), 5.05-5.15 (m,
2H), 5.28 (bs, 1H), 5.35 (d, 1H, $J = 2.76$ Hz), 5.40 (bs,
1H), 5.53 (bs, 3H), 5.65 (d, 1H, $J = 5.60$ Hz), 6.33 (d, 1H,
 $J = 6.04$ Hz, H-1), 7.)-7.3 (m, 68H, Ar-H), 7.3-7.45 (m,
15 9H, Ar-H), 7.45-7.57 (m, 3H), 7.65 (d, 2H, $J = 7.56$ Hz,
Ar-H), 7.75 (d, 2H, $J = 7.48$ Hz, Ar-H), 7.99, 7.96, 7.94
(3d, 6H, $J = 7.52$ Hz, Ar-H.

20

EXAMPLE 19

25

30

Thioglycoside of Nonasaccharide (16): To a solution of
a nonasaccharide 15 (50 mg, 0.0149 mmol) in dry CH_2Cl_2 (1
mL) (suspended with 4 Å molecular sieves (100 mg)), was
added a solution of dimethyldioxirane in acetone (ca 0.08
M, 3 mL). The reaction mixture was stirred for 45 min,
and then solvents were evaporated under a stream of N_2
gas. The residue was dried in vacuum (10 min), and then
dissolved in CH_2Cl_2 (1 mL), and after cooling to -78°C ,
was reacted with EtSH (1mL) and TFAA (5 μL). After 30
min, the mixture was evaporated under a stream of N_2 gas,
and the residue was dried in vaccume. The crude product

5 was dissolved in CH_2Cl_2 (1 mL) and then reacted with
acetic anhydride (0.5 mL) and pyridine (0.5 mL). After
drying for 24 hrs under reduced pressure, the residue was
chromatographed (3:2, Hex:EA) to obtain thioglycoside 16
(60%) as a syrup; ^1H NMR (CDCl_3) δ 0.94 (d, 3H, $-\text{CH}_3$),
10 1.02 (d, 3H, $-\text{CH}_3$), 1.16 (d, 3H, $-\text{CH}_3$), 1.28 (t, 3H, $-\text{CH}_3$),
1.93 (s, 3H, $-\text{COCH}_3$), 2.0 (s, 3H, $-\text{COCH}_3$), 2.04 (s,
3H, $-\text{COCH}_3$), 2.07 (s, 3H, $-\text{COCH}_3$), 2.14 (s, 3H, $-\text{COCH}_3$),
2.71 (m, 2H, $-\text{SCH}_2\text{CH}_3$), 3.1-4.0 (m, several protons),
4.1-5.0 (m, several protons), 4.82 (d, 1H), 4.89 (t, 1H),
15 5.20 (d&m, 2H), 5.35 (d, 1H), 5.45 (d, 1H), 5.50 (bs,
1H), 5.63 (bs, 2H), 5.74 (m, 1H), 7.0-8.2 (m, 90H, Ar-
H);

20 EXAMPLE 20

Sphingosine glycoside (18): To a solution of
thioglycoside 16 (30 mg, 0.0086 mmol) and azidohydrin 17
at 0 °C in dry CH_2Cl_2 : Ether (1:2, 1.5 mL) (suspended
25 with 4 Å molecular sieves (100 mg)) was added MeOTf
(0.0038 mL, 4 eq). The reaction mixture was allowed to
warm up to room temperature. After 24 hrs, the mixture
was diluted with EtOAc (5 mL), filtered through a pad of
silica gel, and washed with a sat'd solution of NaHCO_3 (2
30 x 5 mL). The organic layer was separated, dried (MgSO_4),
and concentrated. The residue was purified by column
chromatography (1:1 Hex:EA) to obtain Sphingosine
glycoside 18 (55%) as a syrup; ^1H NMR (CDCl_3) δ 0.80 (m,

5 9H, 0.85(d, 3H, -CH₃), 0.93 (d, 3H, -CH₃), 1.07 (d, 3H,
-CH₃), 1.18 (bm, 23H, aliphatic -CH₂), 1.33 (bs, 2H) 1.5
(bd, 4H), 1.81 (s, 3H, -COCH₃), 1.94 (s, 3H, -COCH₃),
1.97 (s, 3H, -COCH₃), 2.0 (s, 6H, -COCH₃), 3.1-3.7 (m,
several protons), 3.7-4.1 (m, several protons), 4.2-4.8
10 (m, several protons), 4.82 (d, 1H), 4.89 (t, 1H), 4.97
(d, 1H), 5.1 (m, 2H), 5.37 (m, 4H), 5.47 (d, 1H), 5.53
(bs, 2H), 5.60 (d, 1 H), 5.7 (m, 1H), 7.0-8.1 (m, 95H, Ar-
H).

15

EXAMPLE 21

Amide (protected KH-1 antigen) (18'): To a solution of
azide 18 (15 mg, 0.0039 mmol) in EtOAc (3 mL) was added
20 Lindlar's catalyst (50 mg) and Palmitic anhydride (10 mg,
0.020 mmol). The reaction mixture was stirred at room
temperature under a H₂ atmosphere for 24 h, and then
filtered through a pad of silica gel, rinsed with EtOAc
(20 mL), and concentrated. The residue was purified by
25 chromatography (1:1 EA:Hex) to give amide 18' (85%) as a
syrup: ¹H NMR (CDCl₃) δ 0.79 (m, 9H, 0.84 (d, 3H, -CH₃),
0.92 (d, 3H, -CH₃), 1.06 (d, 3H, -CH₃), 1.17 (bm, 45H,
aliphatic -CH₂), 1.48 (bs, 9H), 1.77 (s, 3H, -COCH₃),
1.90 (s, 3H, -COCH₃), 1.95 (s, 3H, -COCH₃), 1.97 (s, 6H,
-COCH₃), 3.0-3.9 (m, several protons), 4.0-5.0 (m,
30 several protons) 5.51 (bs, 1H), 5.2-5.4 (m, 3H), 5.5 (bs,
1H), 5.6-5.8 (m, 2H); 7.0-8.1 (m, 95H, Ar-H).

KH-1 antigen (1): To a solution of liquid ammonia (5 mL) under N₂ at -78 °C was added sodium (18 mg). To the resulting blue solution was added a solution of protected KH-1 derivative 18' (20 mg, 0.005 mmol) in dry THF (1 mL). After 45 min at -78 °C, the reaction mixture was quenched with absolute MeOH (5 mL). Most of the ammonia was removed in a stream of nitrogen gas. The resulting solution was diluted with MeOH (5 mL) and stirred overnight, and then neutralized with Et₃N·HCl. After stirring for 15 min, the mixture was dried under nitrogen. The crude product was then suspended in DMF (1.0 mL), THF (1.0 mL), and Et₃N (1.0 mL) and treated with Ac₂O (1 mL) and DMAP (cat). After stirring overnight, the reaction mixture was concentrated, passed through a plug of silica gel using EtOAc as an elutant and concentrated. The syrup obtained was dissolved in MeOH (5 mL) and treated with MeONa (5 mg) for 24 h, and then neutralized with Dowex 50-X8. Filtration and concentration gave the KH-1 antigen (70%). An analytical sample was prepared by RP column chromatography, eluting with water-5% methanolic water, followed by lyophilization to obtain 1 as a white powder; ¹H NMR (DMSO) δ 0.95 (m, 3H), 1.1-1.35 (3d, 9H, -CH₃), 1.38 (bm, multiplele protons, alphatic -CH₂), 1.5 (m, 9H), 1.85 (s, 6H, NHCOCH₃), 1.9 (m, 2H), 2.0-2.20 (m, 6 H), 3.0-4.0 (m, Multiple protons), 4.1 (q, 1H), 4.17 (d, 1H, H-1), 4.27 (m, 1H), 4.34 (bm, 1H), 4.41 (d, 1H), 4.6

5 (q, 1H), 4.67 (m), 4.75 (t, 2H), 4.88 (d, 2-3H), 4.97 (d, 1H), 5.36 (m, 1H), 5.56 (m, 1H).

10

EXAMPLE 23

Allyl glycoside (2): To a solution of liquid ammonia (5 mL) under N₂ at -78 °C was added sodium (94 mg). To the
15 resulting blue solution was added a solution of nonasacchride glycal 15 (75 mg, 0.022 mmol) in a dry THF (3 mL). After 45 min at -78 °C, the reaction was quenched with absolute MeOH (5 mL). Most of the ammonia was removed with a stream of nitrogen. The solution was
20 diluted with MeOH (5 mL), stirred overnight, and neutralized with Dowex 50-X8 (846 mg). The resulting mixture was stirred for 15 min, and filtered. The resins were washed with NH₃-MeOH sloution (3 x 20 ml). The filtrates were combined , and dried under a stream of
25 nitrogen gas. The crude product was then suspended in DMF (1.0 mL), THF (1.0 mL), and Et₃N (1.0 mL) and then treated with Ac₂O (1 mL) and DMAP (cat). The reaction mixture was stirred for 24 h, concentrated, passed through a plug of silica gel in EtOAc, and again
30 concentrated. The syrup obtained was dissolved in CH₂Cl₂, then treated with dimethyldioxirane solution in acetone (ca. 0.08 M, 5 mL) at 0 °C under N₂. The mixture was stirred for 45 min, and concentrated under a stream

5 of N₂ gas. The syrup obtained was reacted with allyl
alcohol (5 mL). After 24 h, excess allyl alcohol was
evaporated and the crude syrup was dissolved in MeOH and
treated with MeONa (25% in MeOH, 60 μ L). After 24 h, the
mixture was neutralized with Dowex 50-X8, filtered and
10 concentrated to give allylated nonasaccharide 2 (60%).
An analytical sample was prepared by RP column
chromatography, eluting with water-5% methanolic water,
followed by lyophilization to obtain white powder; ¹H
NMR (D₂O) δ 1.0-1.35 (3d, 9H, -CH₃), 2.0 (s, 6H, -COCH₃),
15 3.3 (bm, 1H, -CHNHAc), 3.4-4.0 (m, multiple protons),
4.08 (bs, 1H), 4.12 (bs, 1H), 4.22 (m, 1H), 4.42 (t, 2H),
4.5 (t, 2H), 4.7 (d, 2H), 4.86 (d, 1H), 5.1 (bs, 2H), 5.26
(bs, 1H), 5.39 (d, 1H) 5.95 (m, 1H, -CHCH=CH₂).

20 PREPARATION OF HEPTA AND KH-1-KLH CONJUGATES BY DIRECT AND CROSS-LINKER METHOD

The allyl glycoside of KH-1 was conjugated to KLH
(Keyhole Lympet Hemocyanin) protein via two different
methods. The first was the direct coupling method which
25 utilized the reductive amination reaction between the
lysines of KLH protein and the aldehyde moiety obtained
by ozonolysis of KH-1 allyl glycoside. This method
typically provides the glycoprotein with around 141
carbohydrate units (KH-1) per KLH.

30 The other conjugation method utilized a cross linker
known as M₂. The same aldehyde of KH-1 antigen utilized
in direct coupling was further derivatized to a suitable
conjugatable form containing M₂ linker. Then the

5 resulting compound was coupled to thiolated KLH protein.
This crosslinker method was highly efficient, providing
the glycoprotein conjugate with around 492 carbohydrate
units (KH-1) per KLH. Figures 12 and 13 describe two
coupling methods.

10 Groups of mice were immunized with both types of
glycoprotein conjugates (KH1-KLH and KH1-M₂-KLH). An
immunological adjuvant QS-21 was co-administered in the
immunization. The antibodies thus elicited were assayed
by ELISA and FACS method. The cross-linked conjugate
15 (KH1-M₂-KLH) showed increased immune response from the
mice, though both types of conjugate effectively elicited
antibodies. Figure 11 describes an alternative synthesis
of KH-1 tetrasaccharide and hexasaccharide.

20 Accordingly, the allyl group in KH-1 or the
heptasaccharide disclosed herein was converted to an
aldehyde group by ozonolysis and linked to -NH₂ groups of
KLH by reductive amination method in the presence of
sodium cyanoborohydride as described for globo H.
(Ragupathi G, et al., *Angew. Chem. Int. Ed. Engl.* 1997,
25 36, 125-128.) In the case of the cross-linker method, the
aldehyde group obtained through ozonolysis was first
reacted with hydrazide of M₂C₆H₄ (4-maleimidomethyl)
cyclohexane-1-carboxyl hydrazide) and reacted with
thiolated KLH as described in Ragupathi G., et al., "A
30 novel and efficient method for synthetic carbohydrate
conjugate vaccine preparation: Synthesis of sialyl Tn-KLH
conjugate using an M₂C₆H₄ linker arm" *Glycoconjugate J.*,
in press. For example, 4 mg of KH-1 allyl glycoside in

5 methanol was stirred at -78°C in a dry-ice/ethanol bath
and ozone gas was passed through the solution for 0 min
under vigorous stirring. The excess of ozone was then
displaced with nitrogen over a period of 5 min. Methyl
sulfide ($100\ \mu\text{l}$) was added and the reaction mixture
10 stirred at room temperature for 2 hours and distributed
equally in two vials. The solvent was removed under a
stream of nitrogen. The resulting white solid was used
directly in the subsequent conjugation steps.

15 Direct Conjugation of KH-1-aldehyde with KLH:

Two mg KH-1 aldehyde was dissolved in 1 ml of
0.1M phosphate buffered saline (PBS) pH 7.2 and 4 mg of
KLH in PBS. Two mg sodium cyanoborohydride was added and
the mixture incubated under gentle agitation at 37°C for
20 48 h. After 16 h, an additional 1.0 mg sodium
cyanoborohydride was added and the incubation continued.
The unreacted KH-1 aldehyde was removed completely with
multiple washes using a Amicon Centriprep with molecular
weight cut-off value 30000 dalton, with 6-7 changes of
25 PBS at 4°C .

Conjugation of KH-1-aldehyde through $\text{M}_2\text{C}_2\text{H}$ to thiolated
KLH:

Preparation of KH-1- $\text{M}_2\text{C}_2\text{H}$

30 Two mg of KH-1-aldehyde was dissolved in 1 ml
of 0.1M sodium acetate buffer pH 5.5, and 4 mg of $\text{M}_2\text{C}_2\text{H}$ in
 $100\ \mu\text{l}$ of dimethyl sulfoxide (DMSO) was added. The
reaction mixture was incubated at room temperature for 15

5 min with gentle stirring. At the end of 15 min 2 mg of solid sodium cyanoborohydride was added and the incubation continued at room temperature for 2 h. Unreacted M_2C_2H was removed in a Sephadex G10 column equilibrated previously with 0.1 M sodium phosphate buffer pH 6.0 containing 5 mM EDTA and eluted with the same buffer. The fractions positive for KH-1 by TLC with orcinol were combined.

Addition of sulfhydryl groups to KLH

15 2-Iminothiolane (2 mg) dissolved in thiolation buffer (50 mM triethanolamine, 0.15 M NaCl, 5 mM EDTA, pH 8.0) was added to 4 mg of KLH and incubated with stirring at room temperature for 2 h. Unreacted 2-iminothiolane was removed by Sephadex G15 column equilibrated previously with 0.1 M sodium phosphate buffer pH 7.2 containing 5 mM EDTA and eluted with the same buffer. Fractions positive for KLH with BioRad protein assay dye reagent were combined. A small portion was used to estimate sulfhydryl groups in the thiolated KLH using Ellman's reagents and cysteine as standard. Riddles P.W., et al., *Anal. Biochem.* 1979, 94, 75-81. The KLH was estimated by a dye method using BioRad dye reagent according to the manufacture's instructions.

30 Conjugation of KH-1- M_2C_2H product and thiolated KLH were mixed and adjusted to pH 7.2 with 0.1M sodium phosphate buffer pH 8.0. The reaction mixture was then incubated at room temperature overnight. The content of the KH-1- M_2C_2H -KLH reaction vial was transferred to a

5 Centriprep concentrator 30 (Amicon: molecular cut-off
30000 Dalton) and unreacted KH-1-M₂C₂H was removed
completely with multiple washes. The conjugate was
checked by HPTLC for the absence of unreacted KH-1 as
mentioned above. The epitope ratios of two batches of
10 conjugate were determined by estimating protein content
by BioRad dye binding protein assay and carbohydrate by
a HPAEC-PAD assay. The epitope ratio of hepta-KLH and
hepta-M₂-KLH was 112/1 and 197/1 respectively. The
epitope ratio of KH-1-KLH and KH-1-M₂-KLH was 141/1 and
15 492/1, respectively.

Table 1. Antibody Titers by ELISA against KH1-KLH

		KH-1-KLH			
		<u>Pre-serum</u>		<u>10 days post 3rd</u>	
	<u>Group</u>	<u>IgM</u>	<u>IgG</u>	<u>IgM</u>	<u>IgG</u>
20	1.1	0	0	100	0
	1.2	0	0	100	0
	1.3	0	0	100	0
	1.4	100	0	300	0
	1.5	100	0	100	0
		KH-1-M ₂ -KLH			
25	2.1	0	0	0	0
	2.2	0	0	900	300
	2.3	0	0	300	300
	2.4	0	0	900	900

5 Table 2. Cell Surface reactivity of KH-1 antibodies on MCF-7 cells by FACS.

	<u>Group</u>	<u>% of cells positive</u>	
		IgM	IgG
10	KH1-KLH		
	1.1	28.4%	14.1%
	1.2	16.9%	18.8%
	1.3	12.9%	11.0%
	1.4	36%	12.3%
15	1.5	35.56%	30.2%
20	KH1-M2-KLH		
	2.1	30.18%	88.1%
	2.2	36.59%	76.2%
	2.3	18.16%	93.1%
	2.4	47.9%	91.9%
	2.5	20.03%	97.9%
25	Mouse pre sera IgM: 1.72%, Mouse preIgG 0.76%, Mab BR96.78.17%		

5 Serological Analysis:

ELISA: Enzyme-linked immunosorbent assays (ELISAs) were performed as described by Livingston, P.O. et al., *Cancer Immunol. Immunother.*, 1989, 29, 179-184, 1989. Serially
10 diluted antiserum was added to wells coated with antigen (0.1 µg) and incubated for 1 h at room temperature. Goat anti-mouse IgM or IgG conjugated with alkaline phosphatase served as secondary antibodies. Absorbance was measured at 414 nm. The antibody titer was defined
15 as the highest serum dilution showing an absorbance 0.1 or greater above that of normal mouse sera.

Flow Cytometry:

Cells from the KH-1-positive breast cancer cell line MCF-7 served as target. Soule, H.D., et al., *J. Natl. Cancer Inst.*, 1973, 51, 1409-1416. Single cell suspensions of 2×10^5 cells/tube were washed in PBS with 3% fetal calf serum and 0.01 M NaN₃ and incubated with 20 µl of 1:20 diluted antisera or mAb BR-96 for 30 min on ice.
20 After washing the cells twice with 3% FCS in PBS, 20 µl of 1:15 goat anti-mouse IgM or IgG-labeled with fluorescein-isothiocyanate (FITC) was added, mixed and incubated for 30 min. After wash, the positive population and mean fluorescence intensity of stained
25 cells were analyzed by flow cytometry (EPICS Profile II, Coulter, Co., Hialeah, FL). Zhang, S. et al., *Cancer Immunol. Immunother.*, 1995, 40, 88-94.
30

5 The mixture was incubated at room temperature for 30 min,
and transferred to an ELISA plate coated with KH-1-
ceramide. ELISAs were performed as described above.
Percentage inhibition was calculated as the difference in
absorbance between the uninhibited and inhibited serum.

10

Immunization of Mice

Groups of mice (CB6F1 female; 6 weeks of age)
obtained from Jackson Laboratory, Bar Harbor, ME, were
immunized subcutaneously with KH-1-KLH or KH-1-M₂C₂H-KLH
15 containing equivalent to 3 µg KH-1 only (the quantity of
KLH varied depending on the epitope density) mixed with
10 µg of immunological adjuvant QS-21, a saponin
derivative from the bark of the *Quillaja saponaria* Molina
tree (Aquila, Worcester, MA) at 0, 1 and 2 weeks and
20 bled 10 days after the third immunization. The presence
of antibody was assayed by an enzyme linked immunosorbent
assay (ELISA) as described in Kensil C.R. et al., *J.*
Immunol., 1993, 146, 431-437, using KH-1 ceramide as
target antigen. The cell surface reactivity of anti-KH-1
25 antibodies was tested on KH-1 positive MCF-7 cells by
flow cytometry assays. The mice vaccinated with KH-1-
M₂C₂H are made the high titer antibody against the
synthetic KH-1 and the antibodies were reacted strongly
on the cell's surface that expressed KH-1 antigens.

30

**Binding of Monoclonal Antibody BR 96 with synthetic KH-1
and other Carbohydrate by Dot-blot Immune Stain:**

5 0.5 μ g KH-1 ceramide and other Le^x antigen and
unrelated antigens were spotted on nitrocellulose strips.
Dot blot Immune staining was performed monoclonal
antibody BR 96 after blocked with 6% bovine serum albumin
in PBS for 1 h and incubated with antibody BR 96 (diluted
10 1:500 in PBS) overnight at room temperature. The strips
were washed with PBS containing 0.05% Tween 20 and
incubated with anti-mouse IgG antibody conjugated with
horseradish peroxidase at 1:200 dilution for 3 h at room
temperature. Then the strips were washed with PBS-0.05%
15 Tween 20 and developed with 4-chloro-1-naphtol-H₂O₂. The
results are summarized in Table 1. The synthetic KH-1
reacted very strongly when compared with other Le^x related
antigens unrelated antigens were failed to react with BR
96 antibody.

20

Table 3. Binding of Monoclonal Antibody Br 96 with KH-1 and other Carbohydrates by Dot-blot.

Carbohydrate	Monoclonal Antibody	
	BR 96 (Le ^y related)	F12 (FucosylGM1)
KH-1 ceramide	very strong (+++)	negative
Le ^y -ceramide	strong (++)	negative
Le ^y -KLH	strong (++)	negative
Globo H ceramide	negative	negative
TF-ceramide	negative	negative
SSEA-ceramide	negative	negative
Le ^y /Le ^b (Ovarian cyst Mucins-Tighe)*	strong (++)	negative
Le ^a /Le ^x (Ovarian cyst mucins-N1)*	weak (+)	negative
Non fucosylated precursor of Lewis*	negative	negative
Le ^a -PAA	negative	negative
Le ^x -PAA	weak (+)	negative
FucGMI	negative	very strong (+++)
GD3	negative	negative

*extracted from patient tissue

Discussion

Human tumors are often marked by the presence of unusual carbohydrate structural motifs. Hakomori, S.,

5 *Cancer Res.*, 1985, 45, 2405; Feizi, T., *Cancer Surveys*,
1985, 4, 245; Lloyd, K.O., *Am. J. Clin. Pathol.*, 1987, 87,
129; Lloyd, K.O., *Cancer Biol.*, 1991, 2, 421. These
carbohydrate domains are encountered as cell-surface bound
glycolipids or glycoproteins. Hakomori, S., *Cancer Cells*,
10 1991, 3, 461. It would be useful for cancer therapy to
achieve some level of immune response by vaccinating cancer
patients with such cell-free carbohydrate domains, obtained
through total synthesis and suitably bioconjugated.
Preliminary synthetic studies have been reported. M.T.
15 Bilodeau, T.K. Park, S. Hu, J.T. Randolph, S.J. Danishefsky,
P.O. Livingston, and S. Zhang, *J. Am. Chem. Soc.*, 1995, 117,
7840; T.K. Park, I.J. Park, I.J. Kim, S. Hu, M.T. Bilodeau,
J.T. Randolph, O. Kwon and S.J. Danishefsky, *J. Am. Chem.*
Soc., 1996, 118, 11488. In addition, the utility of tumor-
20 associated carbohydrate antigens is supported by the
observed establishment of responses to the human cancer
lines by sera of mice immunized with such antigens. G.
Ragupathi, et al., *Angewandte Chemie*, In Press.

 In conducting this project, the important issue of
25 "strategy" in oligosaccharide synthesis is addressed. Of
course, in this field (as opposed to "conventional" natural
product synthesis) the basic building blocks which are
considered to be rather restricted and tend to bear obvious
homology with readily recognized components of the target
30 system.

 From this perspective a plan was pursued which
would build a hexasaccharide (cf. structure 13) so
differentiated in terms of its protecting patterns (see

5 asterisks) as to allow for the unveiling of the three free hydroxyls to serve as α -fucosylation acceptor sites (see structure 13). In this way, the three immunologically defining α -fucose units might be introduced in one concurrent synthetic operation.

10 Assembling the hexasaccharide involved a potentially forbidding network of hydroxyl group functionality. Regarding this, advantages were observed in drawing from a few of the basic principles now well appreciated in the logic of glycal assembly. Bilodeau,
15 M.T.; Danishefsky, S.J., *Angew. Chem. Int. Ed. Engl.*, 1996, 35, 1380.

 Thus, differentiated glycals 4 and 5 are derived from D-glucal by exploiting the reliable reactivity preference of the C₆, C₃ and C₄ hydroxyls (C₆ > C₃ > C₄). More-
20 over, the fashioning of a clean α -epoxide from galactal derivative 3 is known. Also, known (Halcomb, R.L.; Danishefsky, S.J., *J. Am. Chem. Soc.*, 1989, 111, 6661), is the excellent β -galactosyl donating capacity of such an epoxide. Coupling of this epoxide to 4 and to 5, under
25 mediation by a simple reagent (anhydrous zinc chloride), gave 6 and 7, respectively. The C₃' hydroxyl of the lactal derivative 6 was protected as a triethylsilyl derivative. In the resultant structure 8, two of the three sites destined for eventual fucosylation have been distinguished.
30 In a parallel experiment, compound 6 could be converted by acetylation to its C₃' acetate, and overall sulfonamido (2 α) ethanethylation (Griffith, D.A.; Danishefsky, S.J., *J. Am. Chem. Soc.*, 1990, 112, 5811), (1 β) of its glycal linkage

5 (leads to 9 which carries the third eventual fucosylation
center at the site of its TES group). Cleavage of the
carbonate linkage of 7 generated triol 10. Here, advantage
is taken of another well appreciated preference wherein the
glycosyl accepting site in such a triol tends to be at its
10 C₃' hydroxyl acetate (see asterisk). Kameyama, A; Ishida,
H.; Kiso, M.; Haegawa, A.J., *Carb. Chem.*, 1991, 5, 337.
Coupling of 10 and 9 afforded, after cleavage of its cyclic
carbonate and acetate, a pentaol (see structure 11).

15 At this stage, the proposition was pursued in
which the 1,2,3 in the terminal ring D, rather than the 1,3
diol in ring B would serve as the pre-lactosamine acceptor
site with donor 8. This, in fact proved to be the case.
The successful glycosylation was followed by acetylation of
the four remaining hydroxyl groups. This sequence led to 12
20 and thence to 13 as shown.

Thus, it was possible to introduce the three α -L-
fucose residues in one step via donor 14 (Danishefsky, S.J.;
Gervay, J.; Peterson, J.M.; McDonald, F.E.; Koseki, K.;
Oriyama, T.; Griffith, D.A.; Wong, C.-H.; Dumas, D.P., *J.*
25 *Am. Chem. Soc.*, 1992, 114, 8331), thereby affording a 60%
yield of the nonasaccharide. From 15, the sorts of
protocols required to reach 1 and 2 were qualitatively well
precedented. In the case of 2, the chemistry followed very
closely from the methodology developed for the globo-H
30 breast tumor, conjugatable allyl glycoside. M.T. Bilodeau,
T.K. Park, S. Hu, J.T. Randolph, S.J. Danishefsky, P.O.
Livingston, and S. Zhang, *J. Am. Chem. Soc.*, 1995, 117,
7840; T.K. Park, I.J. Park, I.J. Kim, S. Hu, M.T. Bilodeau,

5 J.T. Randolph, O. Kwon and S.J. Danishefsky, *J. Am. Chem. Soc.*, **1996**, *118*, 11488. To reach the naturally occurring glycolipid antigen **1**, a small but useful variant was introduced wherein the pre-ceramide acceptor **17** was coupled to an anomeric thioethyl donor derived from the glycal
10 epoxide. For a review, see: Fugedi, P.; Garegg, P.J.; Lönn, H.; Norberg, T.; *Glycoconjugate J.*, **1987**, *4*, 97; Lönn, H., *Carbohydr. Res.*, **1985**, *139*, (105) 115; Lönn, H., *Carbohydr. Chem.*, **1987**, *6*, 301.

The structures of the final products **1** and **2** were
15 fully substantiated by mass spectroscopy, self consistent nmr analysis, and in the case of **1**, correspondence with the available published data. Nudelman *J. Biol. Chem.*, **1986**, *261*, 11247.

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